Evaluation of Anti-Epileptic Activity of Ethanolic Extract of Leaves of *Cassia alata* Linn. By Maximal Elecroshock (MES) and Isoniazid induced convulsions on mice

Dissertation submitted to

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In partial fulfilment for the award of the degree of

MASTER OF PHARMACY IN PHARMACOLOGY

By

Register No: 261425011

UNDER THE GUIDANCE OF

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Certificate of Botanical Identification

Certified that the following plant drug taken up by Mr. **J.Ganesh Kumar**, II year M.Pharm scholar for his Dissertation work at C.L Baid Mehtha college of Pharmacy, Chennai, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Taxonomical and Microscopical methods as

Cassia alata Linn. (Caesalpiniaceae), Leaves

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DECLARATION

I do hereby declare that the thesis entitled **"Evaluation of Anti-Epileptic Activity of Ethanolic Extract of Leaves of Cassia alata Linn. By Maximal Elecroshock (MES) and Isoniazid induced convulsions on mice"** was carried out by me under the guidance and supervision of **Dr. P. Amudha M.Pharm., Ph.D.,** Asst. professor, Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai-97. The work embodied in the thesis is original, and is not submitted in part or full for any other degree of this or any other university.

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LIST OF ABBREVATIONS

Ach	Acetylcholine
AD	After discharge
AED	Anti-epileptic drug
ALT	Alanine aminotransferase
ALP	Alkaline phosphatise
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
AS	Audiogenic seizures
AST	Aspartate amino transferase
Ca^{+}	Calcium ion
CAT	Computerized axial tomography
CK	Creatine kinase
Cl^{-}	Chloride ion
CNS	Central nervous system
CPCSEA	Committee for the purpose of Control and Supervision of Experiments on Animals
CSF	Cerebrospinal fluid
CT	Compurterized tomography
$CuSO_4$	Copper sulphate
EEG	Electroencephalogram
EECA	Ethanolic extract of *Cassia alata*
E/I	Excitation / Inhibition
GABA	γ-amino butyric acid
GAT	GABA transporter

GAD	Glutamic acid decarboxylase
Glu	Glutamate
H_2O	Water
H_2O_2	Hydrogen Peroxide
HPLC	High performance liquid chromatography
HPTLC	High pressure thin layer chromatography
Hcl	Hydrochloric acid
i.p	Intraperitoneal
ILAE	International League Against Epilepsy
ICES	International Classification of Epileptic Seizures
IPSPs	Inhibitory postsynaptic potentials
Kg	Kilogram
KOH	Potassium hydroxide
K^+	Potassium ion
LDH	Lactate dehydrogenase
LD_{50}	Lethal Dose – 50%
mA	Milli ampire
MEG	Magenetoencephalography
MES	Maxiamal electric shock mothd
MDA	Malonaldehyde
mmol/dl	milli mole per decilitre
mol. Wt	Molecular weight
Mg	Milligram
mg/dl	Milligrams per decilitre

MRI	Magnetic resonance imaging
mL	Milliliter
MSI	Magnetic source imaging
N	Normality
NAD+	Nicotinamide adenine dinucleotide
Nm	Nano meter
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
NO	Nitric oxide
O_2-	Superoxide
OECD	Organization for Economic Co-operation and Development
PET	Positron emission tomography
POD	Peroxidase
p.o	Per orally
PTX	Picrotoxin
RH	Room humidity
SE	Status epilepticus
Sec	Seconds
SPECT	Single photon emission computed tomography
STR	Strychnine
TGB	Tiagabine
TLC	Thin layer chromatography
TLE	Temporal lobe epilepsy
VGB	Vigabatrin
WHO	World Health Organization

%	Percentage
µ	Micro
µl	Microliter
ºC	Degree Celsius

Introduction:

Epilepsy is a chronic non-communicable disorder of the brain that affects people of all ages. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally. Nearly 80% of the people with epilepsy live in low and middle-income countries. People with epilepsy respond to treatment approximately 70% of the time. About three fourths of people with epilepsy living in low and middle income countries do not get the treatment they need.[1]

Epilepsy is the second common neurological disorder in India. In the 70% of the population with epilepsy there is high prevalence in children about 0.8% and it can affect 1 person in 200 of people.[2]

According to WHO people with epilepsy disorder in the growing countries, not getting proper treatment.[3]

It is a neuropsychological disorder which occurs due to over discharge of neurotransmitter substance. Some of reasons are common in epilepsy due to scaring tendency, chemical and hormonal imbalance, tumours or brain damage. It also includes sensitivity and low threshold of brain to epilepsy produces that epilepsy. And it can affect the people at any age, sex, and race at any part of the life.[4]

The common cause of epilepsy is exactly not known. It may due to various reasons including trauma during birth process, head injury, childhood fevers, brain tumours, meningitis or drug induced. Genetic inheritance of single gene defects account for epilepsy in a small percentage of patients.

A seizure usually occurs in the duration of few minutes mostly the persons recovers quickly. Despite regular treatment, 20-30% of the epileptic patients continue to have epilepsy seizure and they required to treated with two or more antiepileptic drugs.[5]

The alternative drug therapy for the management of this disease can be by the use of medicinal plants and their active principles. Control to seizures numerous conventional drugs came into existence. Most of the epileptic patients need polytherapy of conventional anticonvulsants and still not 100% cured.[6]

Antiepileptic drug therapy is the mainstay of treatment for most patients with recurrent seizures. The antiepileptic drugs act by three basic mechanisms,

- Increased activity of GABA
- Decreased glutamate activity and
- Modification of ionic conductance.

Approximately one-third of the patients develop refractory epilepsy requiring treatment with a combination of two or more antiepileptic drugs.[7]

So now a day we are focussing herbal medicines to get relief with the symptoms of epilepsy. The medicinal plants for the study were selected in such a way that their availability is maximized in many parts of the world.[8]

World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care.[9]

The literature reveals that plants contain a large diversity of natural products that have demonstrated antiepilepsy are like *Erythrophleum ivorense*, *Hypericum perforatum*, *Piper methysticum*, *Actaea racemosa*[10-13]

The plant *Cassia alata* Linn belongs to family Caesalpiniaceae. It is commonly known as ringworm senna. It is used for treatment of ringworm, scabies, ulcers, and skin disease such as pruritis, eczema and itching. In the present study, antiepileptic potential of alcoholic extracts of leaves of *Cassia alata* have been evaluated.

2. LITERATURE REVIEW

2.1 DEFINITION:

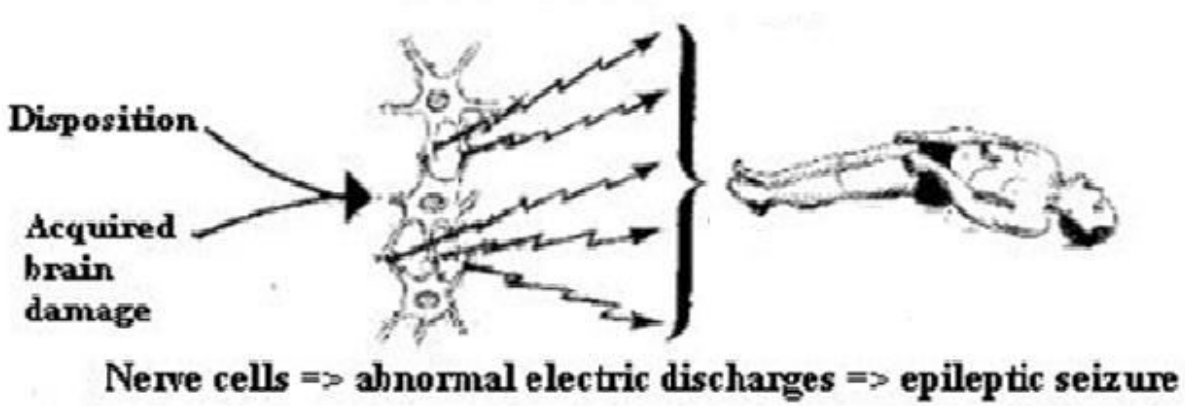
Epilepsy is a chronic disorder of the brain that affects people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized), and is sometimes accompanied by loss of consciousness and control of bowel or bladder function.

Epilepsy is a neurological disorder – a physical condition – which causes sudden bursts of hyperactivity in the brain. This hyperactivity produces "seizures" which vary from one person to another in frequency and form. A seizure may appear as

- Brief stare
- Change of awareness
- convulsion.[14]

Seizure episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. Seizures can also vary in frequency, from less than 1 per year to several per day.

Figure 1:



2.2 EPIDEMIOLOGY

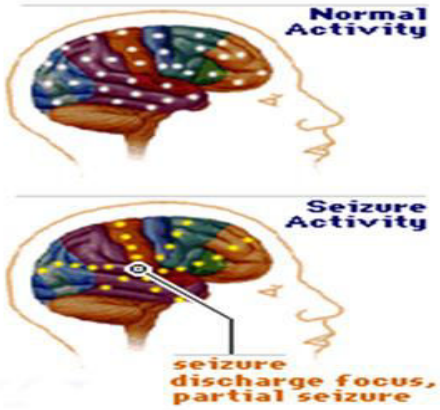
One seizure does not signify epilepsy (up to 10% of people worldwide have one seizure during their lifetime). Epilepsy is defined as having 2 or more unprovoked seizures.

Epilepsy is one of the world's oldest recognized conditions, with written records dating back to 4000 BC. Fear, misunderstanding, discrimination and social stigma have surrounded epilepsy for centuries. This stigma continues in many countries today and can impact on the quality of life for people with the disorder and their families.[1]

About 50 million people worldwide have epilepsy and 90% of them 2 are from developing countries.[14]

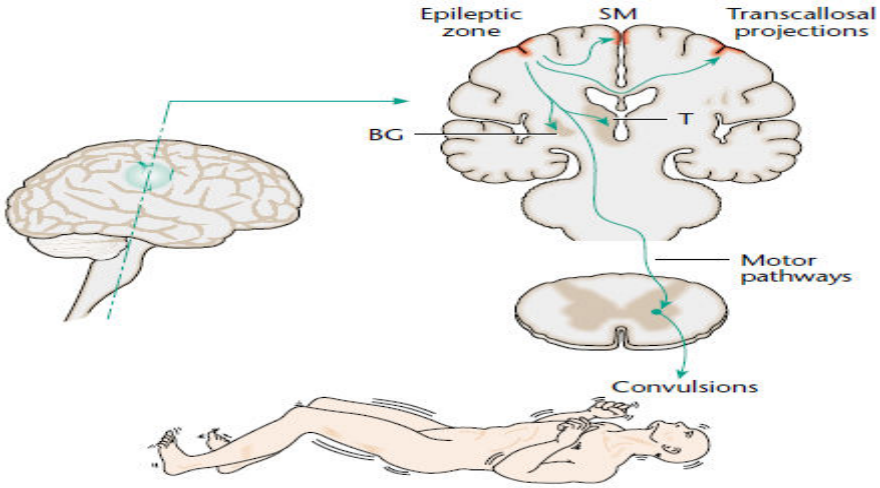
Some medical conditions may cause seizures, these include: febrile seizures (caused by high fever in children), withdrawal seizures, and seizures caused by poisoning, allergic reaction, infection, or an imbalance of body fluids or chemicals (low blood sugar). These are not considered to be forms of epilepsy[15]

Figure 2:



Incidence is a measure of the number of new cases of a medical condition that occur in the population during a measured amount of time, usually one year. There are approximately 200,000 new cases of seizures and epilepsy that occur each year. The lifetime incidence of seizures is about 5% to 10%. Prevalence is the total number of existing cases of a disease in a specific population at a stated point in time. The prevalence of epilepsy is 2.7 million Americans of all ages. Males are somewhat more likely to have seizures than females. Approximately 10% of Americans will have a seizure in their lifetime. Every year about 300,000 people have the first seizure in their life time.[16]

Figure 3:



Epileptic seizures vary in severity and frequency, some people may experience no more than 2-3 seizures during their entire lifetime, others will have several seizures in one day.[17]

2.3 ETIOLOGY:

In approximately 60-75% of all cases, there is no known cause. Of the remaining cases, there are a number of frequently identified causes.

Identifiable Causes

- Brain injury to the fetus during pregnancy
- Birth trauma (lack of oxygen)
- Head trauma (car accident, sports injury, shaken baby syndrome)
- Substance abuse
- Alteration in blood sugar (hypoglycemia)
- Other metabolic illness (hypocalcemia)
- Stroke[14]
- Unknown causes
- Vitamin deficiency
    - ❖ (e.g., pyridoxine deficiency)

- Hypoxic-ischaemic encephalopathy
- Hypotensive syndromes (Shock, Stokes- Adams attacks, vasodepressor syncope)
- Drugs- Therapeutic (e.g., penicillins imepenem, isoniazid, phenothiazines, meperidine, theophylline, cyclosporine 506 [tacrolimus]
- Drugs- Recreational (e.g., cocaine)
- Alcohol withdrawal
- Sedative drug withdrawal
- Environmental toxins (e.g., lead, mercury, arsenic, strychnine, thallium)
- Hypertensive encephalopathy
- Endocrine
    - ❖ Thyrotoxicosis
    - ❖ Hypothyroidism
- Eclampsia
- Organ Failure:
    - ❖ Hepatic failure
    - ❖ Renal failure
- Fever
- Metabolic
    - ❖ Hypoglycemia
    - ❖ Hyponatremia/hypernatremia
    - ❖ Hypocalcemia
    - ❖ Hypomagnesemia
    - ❖ Alkalosis
- Cerebral infections
    - ❖ Meningitis
    - ❖ Encephalitis
    - ❖ Abscess
    - ❖ Neurosyphilis
- Toxins/poisons
- Brain tumour/lesion[18]
- Cerebral vascular diseases

- Thrombosis
- Embolism
- Hemorrhage
- Vasculitis

Figure 4:

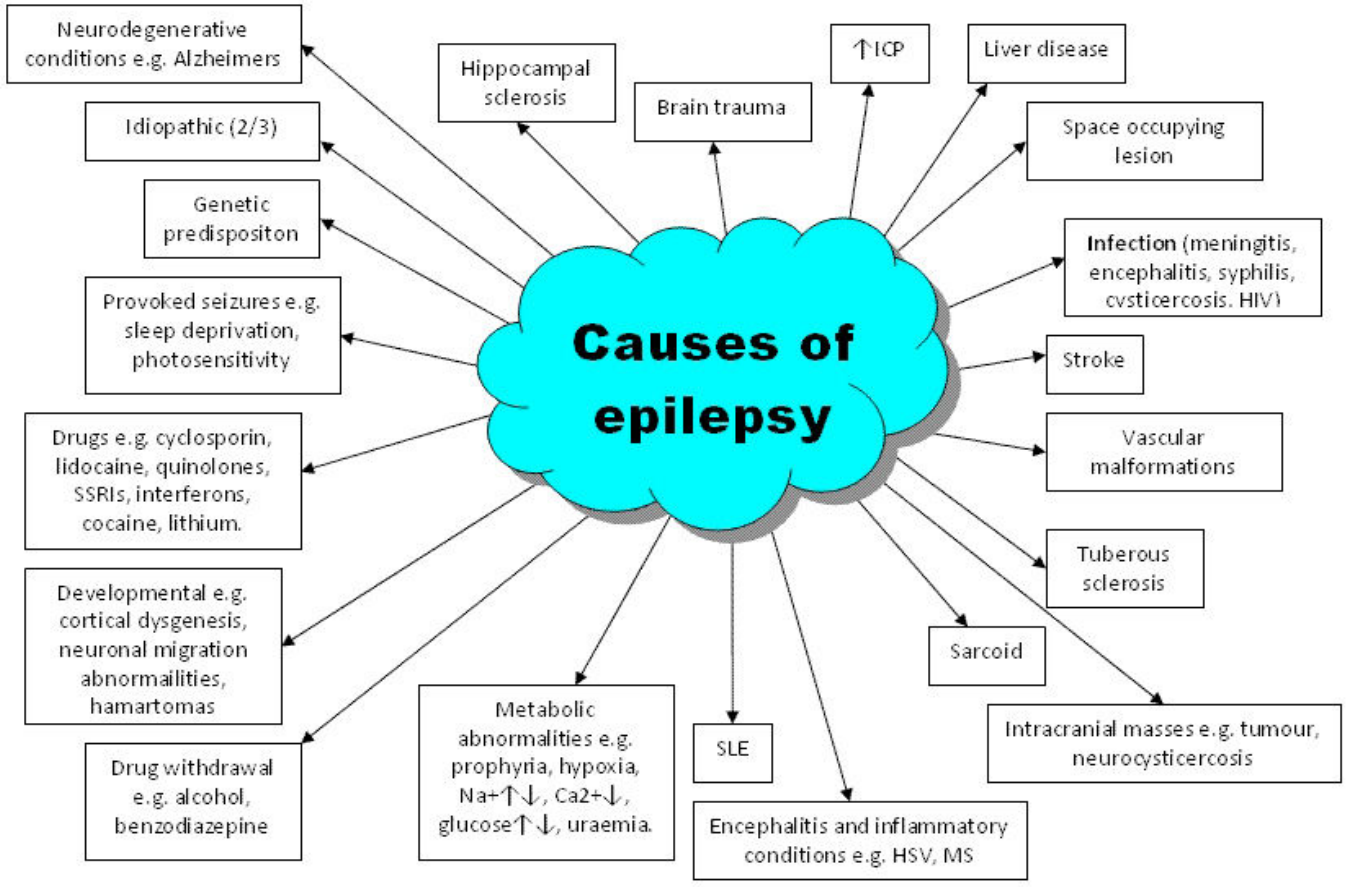
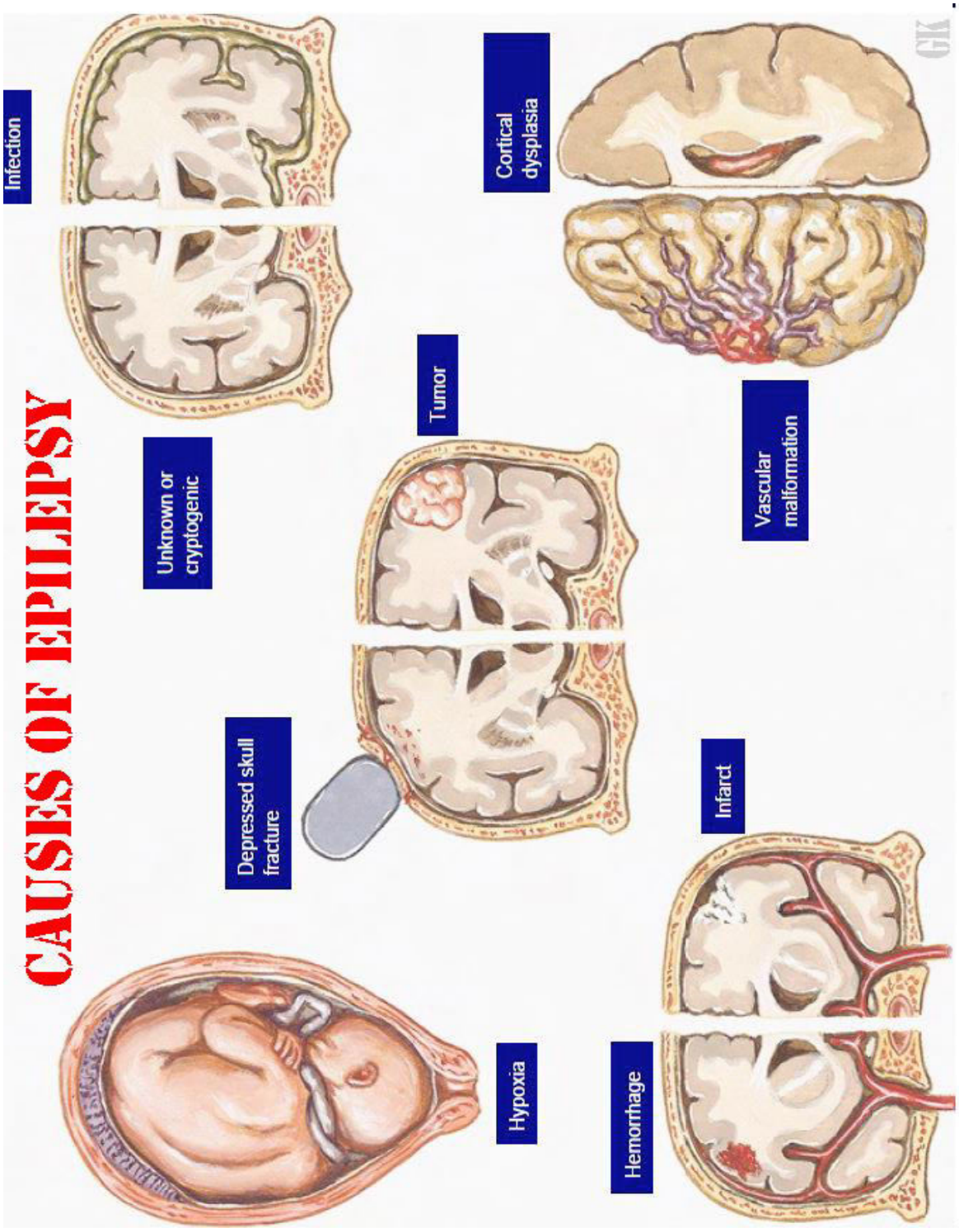


Figure 5:


CAUSES OF EPILEPSY
Infection
Cortical dysplasia
Tumor
Unknown or cryptogenic
Vascular malformation
Depressed skull fracture
Infarct
Hypoxia
Hemorrhage
GK

2.4 Diagnosis:

1. CT scan
2. EEG
3. MEG/MSI
4. MSRI
5. PET
6. MRI
7. Functional MRI
8. SPECT[14]

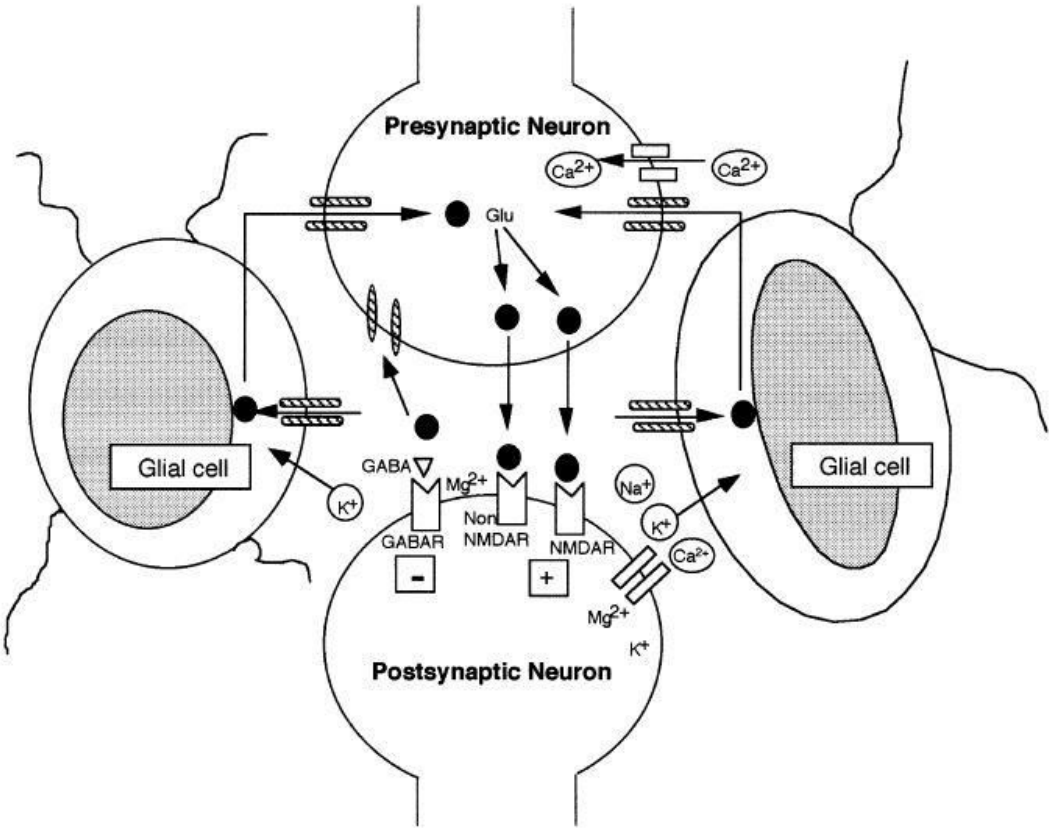
2.5 Pathophysiology

Epileptic seizures arise from an excessively synchronous and sustained discharge of a group of neurons. The single feature of all epileptic syndromes is a persistent increase of neuronal excitability. Abnormal cellular discharges may be associated with a variety of causative factors such as trauma, oxygen deprivation, tumors, infection, and metabolic derangements. However, no specific causative factors are found in about half of the patients suffering from epilepsy[19.]

A seizure is produced when neurons within an area of the brain are activated in an unusually synchronous manner. Focal activation of a group of neurons may subsequently spread to involve nearby or distant neurons in an abnormal activation pattern. Any event, or combination of events, that disturbs the delicate balance between neuronal excitation and inhibition can produce a seizure[20]. Many different cellular or biochemical changes such as alterations in ion channel function, neurotransmitter level, and neurotransmitter receptor function, or energy metabolism may affect the excitability of neurons and produce seizures. In general, depolarization is mediated by synaptic currents generated by the excitatory neurotransmitters glutamate and aspartate[21]. Neuronal synchronization occurs through local enhancement of excitatory circuits. An increase in synaptic efficacy is thought to be due to recruitment of *N*-methyl-D-aspartate (NMDA) receptors[22]. As more NMDA receptors are activated, further depolarization occurs, additional calcium enters the cell, and excitability is enhanced. As these excitatory processes increase, there may be a simultaneous reduction in the

activity of inhibitory circuits that are down-regulated during high-frequency activation[23]. Neurons can also be synchronized by extracellular currents that may reflect changes in the perineuronal environment, such as local edema, or changes in the extracellular potassium, calcium, or magnesium concentration[24]. Finally, neurons may also be synchronized by local ephaptic (non-synaptic) contacts, which facilitate the development of excitatory circuits[25, 26].

Fig 6:



Neuroexcitability: The presynaptic and postsynaptic neuron. Excitatory amino acids are released from the presynaptic terminal and act on postsynaptic NMDA and non-NMDA receptors (NMDAR) to cause excitation. GABA is an inhibitory neurotransmitter and acts on postsynaptic GABA receptors (GABAR). The glial cells play a central homeostatic role in the control of neuroexcitation by controlling extraneuronal potassium concentration and by removing excitatory neurotransmitters such as glutamate (Glu). Neuronal excitability may also be influenced by ions such as magnesium.

blood–brain barrier due to infection, hypoxia, or alterations in cerebral blood flow autoregulation may allow passage of drugs and toxins into the CNS, thus influencing neuronal excitability (Fig. 2). Changes in the integrity of the blood–brain barrier may also influence homeostasis within the neuronal microenvironment that is normally tightly regulated by the glial cell (Fig. 1). For

example, glial cells normally maintain a low concentration of extracellular Potassium[27]. Interruption of the blood–brain barrier may directly cause glial cell dysfunction or change the extracellular environment beyond its regulatory capacity. Glial cell dysfunction may lead to seizures by permitting a high ratio of extracellular to intracellular potassium, which depolarizes the neuronal membrane and increases neuronal excitability[28].

2.6 International classification:

The International League Against Epilepsy (ILAE) first published a classification system in 1960. The last official update for seizures was published in 1981, and the last official update for the epilepsies was in 1989. By definition, epilepsy is diagnosed after a patient has two or more unprovoked seizures. The 1981 and 1989 updates form the officially accepted classification system, although there continues to be efforts to develop a clinically meaningful revision to the current system. A report in 2010 by the ILAE Commission on Classification and Terminology recommended that changes be made in the current conceptualization, terminology, and definitions of seizures and epilepsy. This chapter will focus primarily on the currently accepted standard based on the 1981 and 1989 reports, and discuss the recommendations of the 2010 ILAE report.[29]

GENERALISED SEIZURES:

1. Absence seizures (petit mal)
2. Myoclonic seizures
3. Clonic seizures
4. Tonic seizures
5. Tonic clonic seizures (grand mal)
6. Atonic seizures (drop attacks)
    - Typical absences
    - Atypical absences[30]

PARTIAL SEIZURES:

1. Simple partial seizures (consciousness not impaired)
   a. With motor symptoms
   b. With sensory symptoms
   c. With autonomic symptoms
   d. With psychic symptoms
2. Complex partial seizures (with impaired consciousness)
   a. Simple partial seizures followed by impairment of consciousness
   b. With impairment of consciousness at seizure onset
3. Partial seizures evolving to secondarily generalized seizures
   a. Simple partial secondarily generalized
   b. Complex partial secondarily generalized
   c. Simple partial evolving to complex partial evolving to generalized.

Sub categories:

The four main subcategories of seizure are:

- Generalized seizures , affects entire brain
- Partial seizures , affect the part of the brain
- Non-epileptic seizures, caused by other thing such as diabetes, high fever, or other relevant causes.[31]

I. GENERALISED SEIZURES:

Generalized seizures (absence, atonic, tonic clonic and myoclonic) involve both sides of the brain, while partial (simple and complex) seizures involve only a part of the brain.

1. Absence seizures:

Formerly known as petitmal seizure. These seizures usually last from 2 to15 seconds and may occur just a few times a day, or more than 100 times in a single day. They usually present as bland staring, which one might mistake for daydreaming, physical automatisms, such as lip smacking, fumbling or picking at clothes, or twitching of facial or body muscles. Afterward, the person will likely have no memory of what happened while he or she had the seizure. A lot of

people won't recognize absence seizures as seizures. They occur mostly among children, starting between the age of 4 and 12. They rarely begin after age 20. Most children with typical absence seizures are otherwise normal.

2. Absence seizures with special features:

Eyelid myoclonia with associated interruption of awareness is the characteristic seizure type in Jeavon's syndrome. Myoclonic seizures (discussed below) can also be associated with momentary loss of awareness.[32]

3. Generalized tonic clonic seizure:

This type of seizure formerly known as grandmal seizures; also known as convulsions or convulsive seizures. When most people think of seizures or epilepsy, they're thinking of this type of seizure. When a person has a tonic clonic seizure, his or her arms and legs will first stiffen. This is the tonic stage. His or her limbs and head will then begin jerking, which is the clonic phase. Like all seizures, Generalized Seizures these can vary, mostly with people experiencing either the tonic or clonic phase by itself. During the seizure, the person might bite their tongue or the inside of his or her mouth, experience incontinence, or even decrease or cease his or her breathing (in this case, his or her breathing should return to normal during the tonic (jerking) portion of the seizure). Afterward, the person will likely be confused, not remember what happened, need to sleep for a while and might have a headache. Depending on the person, it can take them from minutes to hours to fully recover. For people with tonic clonic seizures, it is especially important to make sure that those who spend much time around them know correct seizure first aid. Some people bite their tongue or lose control of their bladder during the seizure.[33]

During this seizure a person will usually emit a short cry and fall to the floor. This cry does not indicate pain. [14]

In its classic form, this seizure is a sequence of events that often begins with bilateral myoclonic jerks. This is then followed by a tonic contraction of the extremities and axial trunk muscles, resulting in extension of the neck and extension of the extremities. The tonic contraction of the diaphragm and abdominal and chest wall muscles against the contracted glottis

causes the characteristic tonic cry. The patient may become cyanotic during this phase. The generalised tonic activation can only be sustained for a short period of time.[32]

4. **Myoclonic seizures**:

These seizures cause parts of a person's body to jerk for instance, his or her arm or leg might suddenly twitch. If you've ever had a foot twitch suddenly when you're asleep, that would be a lot like a myoclonic jerk (but it does NOT mean you have epilepsy. The jerking of feet while you're asleep is a type of non-epileptic behavior). Someone who has myoclonic seizures might be thought of as clumsy. First aid is usually not required for myoclonic seizures, but if it is your first seizure of this type, you might want to visit a physician to determine what is causing it.

5. **Atonic seizures:**

It is also known as drop attacks, or astatic or akinetic seizures. Atonic seizures make your muscles suddenly relax. This makes you fall down without warning[33]. These seizures make a part, or all, of a person's body suddenly go limp. This means that the person's head might suddenly drop, or he or she could slump down or even totally collapse, dropping to the floor (thus the name drop attack). Because of the sudden and complete nature of these types of seizures, they can be dangerous to the person having one. This is why children and adults who experience these types of seizures sometimes wear protective headgear. To make matters worse, these types of seizures often won't respond to epilepsy medications. No first aid is needed for a person having an atonic seizure, unless the person has hurt himself or herself during a fall, or if this is the first time he or she has had an atonic seizure.

II. PARTIAL SEIZURES:

(Simple partial seizures and complex partial seizures) are the most common type of seizures. They occur when only a part of one side of the brain is affected. With these seizures, the activity can start in one place in the brain, and then move to another, or it could just stay in the one area. Partial seizures affect whatever function the part of the brain they're occurring in controls. If the seizure happens in the brain's speech area, a person's ability to talk will be affected. Almost any sort of movement or feeling can be a part of a partial seizure. If the seizure starts off as a partial seizure, then spreads to include the entire brain, it's referred to as a partial seizure secondarily.[31]

Restriction for people with only simple partial seizures depends on the specific seizure manifestations (and, for driving, on regulations in a particular state). Partial onset seizures may progress to secondarily generalized seizures.[34]

Partial seizures manifest themselves in many different forms, depending on which area of the cortex is involved in the onset and spread of the ictal discharge. Partial seizures originate from a focal area of cerebral cortex and may spread to other cortical regions either unilaterally or bilaterally. A partial seizure may manifest with motor signs, autonomic symptoms, somato-sensory or special sensory symptoms, or psychic symptoms. The term aura comes from the Latin word "breeze" and is synonymous with a simple partial sensory or psychic seizure. An aura often reflects the location of the seizure onset zone, although there is exceptions.[29]

Simple partial seizures:

A person having a simple partial seizure will often stay awake and aware throughout the seizure but, although they know what's happening, are unable to speak and/or move until the seizure is over. Depending on the part of the brain affected during the seizure, the person might move uncontrollably. For instance, they might twitch, roll their eyes, shake their hands or feet or blink rapidly. These movements might start slowly, and then increase in rapidity or in the parts of the body involved. What starts as a hand bobbing up and down might evolve to an arm moving up and down, or even half of the body moving in a rhythmic manner. If the seizure affects a different part of the brain, the person's emotions or senses might be affected instead. So they might have a feeling of déjà vu or that something terrible is about to happen. They might also suddenly become very angry or very happy. If their senses have been affected, they might hear, smell, taste, feel or see something that is not actually there. They might feel a breeze when they're indoors; hear hearing, buzzing or talking that isn't happening; think something is narrower or wider or closer or farther than it is. They could even hallucinate something from their past. They might even burst out laughing or crying, and, like movement-oriented seizures, these seizures might start small or mildly and then increase in intensity.

Auras are simple partial seizures that may precede loss of consciousness (progression to a complex partial or secondarily generalized seizure). People with particularly vivid or disabling complex partial seizures may also use this term to refer to the earliest and mildest ictal

symptoms. Many patients recognize the aura as a 'warning' that a larger seizure is about to occur. The aura may allow the patient to avoid injury or embarrassment by seeking a safe place to sit or lie down before the larger seizure occurs.[34]

Complex partial seizures:

These seizures affect a greater part of the brain than simple partial seizures and they also affect consciousness. Although they can affect any part of the brain, they generally take place in one of the brain's two temporal lobes. Because of this, people prone to complex partial seizures are often said to have temporal lobe epilepsy (TLE). Usually, when a person has a complex partial seizure, they'll stop what they're doing and stare blankly at nothing in particular. They'll stop interacting with their environment and with other people. (During simple partial seizures they can interact with other people). They will then often start chewing, picking at their clothes, mumbling nothing in particular, performing repetitive motions, or any combination of these simple, unorganized movements. During complex partial seizures, people might appear conscious and normal because they'll usually move about and remain standing with their eyes open—but they'll be experiencing an altered consciousness. In other words, it'll be rather like they're dreaming or in a trance. If they talk, which they might be able to, they'll likely not make sense—and they won't be able to respond appropriately to others.[31]

Complex partial seizures impair consciousness and occur in all age groups. Typically, staring is ac- companied by impaired responsiveness, cognitive function, and recall, although some degree of responsiveness may be preserved (e.g., orienting toward a stimulus). Automatic movements (automatisms) are common and involve the mouth (e.g., lip smacking, chewing, swallowing), upper extremities (e.g., fumbling, picking), vocalization/verbalization (e.g., grunts, repeating a phrase), or complex acts (e.g., shuffling cards). More dramatic automatisms occasionally occur (e.g., screaming, running, disrobing, and pelvic thrusting). Complex partial seizures usually last from 15 seconds to 3 minutes. After the seizure, post-ictal confusion is common, usually lasting less than 15 minutes, although other symptoms, such as fatigue, may persist for hours.[34]

PHASES IN SEIZURES:

Pre-ictal or prodrome:

This is the time before the seizure. It can last from minutes to days and make people act and feel differently. Not everyone experiences something at this stage of a seizure. Some people who do experience a pre-ictal stage use it as a warning so they can prepare for the seizure. Of course, sometimes all it does is make you not feel very good for a few hours or a day before the seizure, while it doesn't give you much of a clue about when the seizure will actually take place. Many people have an aura before a seizure. Technically, an aura is a simple partial seizure. Realistically, an aura might make you see, smell, hear or taste something for no reason. It can even just make you a bit nauseous, give you a weird feeling in your stomach, cause a ringing in your ears, or even just give you a funny feeling or a sense of déjà vu.[35]

Status epilepticus:

Seizures are almost always self-limiting. Rarely one may follow another in close succession (without complete recovery in between seizures), or the ictal activity may be ongoing. Status epilepticus has been traditionally defined as ongoing seizure activity for 30 minutes or more. However, most seizures self-limit within five minutes or less. From a pragmatic point of view, a seizure that lasts longer than five minutes often warrants pharmacological intervention.

Status epilepticus refers to the situation in which a patient suffers from 2 or more generalised epileptic seizures without regaining consciousness between the seizures, or suffers from continuous partial seizures without clear cessation of epileptic activity between the seizures. It is a medical emergency.[36]

Ictal:

This is the actual seizure. During this time there will be actual physical changes in the person's body. After all, it's at this point that the electrical storm in the person's brain thunders to life. If the person with epilepsy were to be hooked up to any medical devices at this point, they'd show cardiovascular, metabolic and EEG changes. A lot of these changes will help a neurologist determine the seizure's type and point of origin, both of which are very important in

treating the epilepsy. We'll cover the different types of seizures a person might experience when they're ictal elsewhere in this pamphlet.

Interictal:

This is the time between seizures. A lot of people with epilepsy, including more than half of all people with temporal lobe epilepsy, suffer emotional disturbances between seizures. These disturbances range from mild fear to pathological levels of anxiety and depression. However, anxiety and depression are by far the most common, and these interictal problems are often more incapacitating and difficult to control than the seizures themselves.

Co/ Inter-ictal:

This is the final phase, the often slow recovery period after a seizure. It can last from minutes to hours and vary quite a bit, partly depending on the type of seizure experienced, the intensity of it, and how long it lasted. It might leave the person feeling tired and/or bewildered, among other things, with a change in his or her consciousness or behavior. Sometimes symptoms from this phase can help doctors diagnose the part of the brain involved in the seizure. Many people will not remember anything that happened during the seizure.[31]

Convulsive status:

This is a state of recurrent tonic-clonic seizures without recovery of consciousness between attacks. It represents a medical emergency with a high morbidity and mortality. Status may occur in approximately 3% of people with epilepsy but it is most common in patients with severe epilepsy who are non-compliant with drug therapy. It may also occur in alcohol withdrawal, in acute meningitis or encephalitis, and in acute metabolic disturbances.

Non-convulsive status: This term is used imprecisely for the following two very different scenarios:

a) Motor manifestations in convulsive status inevitably cease at some point, however the cerebral cortex may continue to generate ictal discharges (no longer convulsive status epilepticus). This represents the most severe form of status, with ongoing excitotoxicity on a cellular level and high morbidity and mortality from a clinical perspective.

b) Ictal activity that from the onset was not associated with motor manifestations. Usually, the leading symptom is a change in the patient's cognitive state (confusion, disorientation with subsequent amnesia). This kind of status epilepticus is thought to have focal origin, though this may no longer be evident once the ictal activity has been ongoing.

Focal status:

The ongoing seizure activity that defines status epilepticus may be restricted to a confined brain area. In this setting, the ictal symptoms reflect the cortical area affected (e.g. aura continua, aphasia). One classic example is epilepsia partialis continua of Kojevnikov. This refers to repetitive jerking of muscles or muscle groups in the face, arm or leg, originally described in association with epidemic encephalitis in Russia. Nowadays, the most common aetiologies are vascular disease, Rasmussen encephalitis, and tumours. Relative frequency of seizure type's data on the relative frequency of seizure types is unsatisfactory, and is largely based on populations of patients with relatively severe epilepsy, including large numbers of patients with partial epilepsies. Furthermore, the milder the epilepsy the more difficult it is to determine on clinical and electroencephalographic grounds whether it is of primary generalised or partial type. With these restrictions in mind, most series would suggest that approximately one-third of epilepsies may be of a generalised type, while two-thirds are partial, most commonly with a temporal lobe origin.

Response to Medication:

On average, 70% of seizures are successfully controlled with one anti-epileptic medication. The remaining 30% of seizures are, thus far, resistant to medications.

History of Medications:

For more than 100 years, various kinds of medications have been used to treat seizure disorders. 1861 – Bromides the first medication used to control of seizures. Side effects were severe. 1912 – Phenobarbital Effective, but sedating. 1936 – Phenytoin Known as the "miracle drug" of its day. Today – Many new medications are available, including a number approved since 1990. The Future – Research continues to be done in an effort to find a safe, effective anti-convulsant. [14]

2.7 SYMPTOMS:

Almost all seizures are relatively brief, lasting from a few seconds to a few minutes. Most seizures last from 1 to 2 minutes. When a seizure stops, people may have:

- Headache
- Numbness or tingling (pins and needles) in a specific body part
- Confusion
- Sore muscles
- Unusual sensations (taste, smell, etc.)
- Extreme tiredness
- Loss of bowel or bladder control (soiling or wetting).[37]

2.8 DISEASES, SYNDROMES, AND EPILEPSIES

Disease versus syndrome:

Although there is reason to distinguish the concepts of disease and syndrome, these terms are not consistently used in medicine. Ultimately, it was decided not to insist on the disease–syndrome distinction in referring to the epilepsies at this time, although either or both terms have been and will continue to be used depending on the context and custom. Instead, there are at least three or four groupings that may be invoked in this context and as described below: Electro-clinical syndromes: Henceforth, the use of the term ‘‘syndrome’’ will be restricted to a group of clinical entities that are liably identified by a cluster of electro clinical characteristics. Patients whose epilepsy does not fit the criteria for a specific electro-clinical syndrome can be described with respect to a variety of clinically relevant factors (e.g., known etiology and seizure types). This does not, however, provide a precise (syndromic) diagnosis of their epilepsy.

Constellations:

In addition to the electro clinical syndromes with strong developmental and genetic components to them, there are a number of entities that are not exactly electro-clinical syndromes in the same sense but which represent clinically distinctive constellations on the basis of specific lesions or other causes. These are diagnostically meaningful forms of epilepsy and may have implications for clinical treatment, particularly surgery. These include mesial temporal lobe epilepsy (with hippocampal sclerosis), hypothalamic hamartoma with gelastic seizures, epilepsy with hemi convulsion and hemiplegia, and Rasmussen ''syndrome.'' Age at presentation is not a defining feature in these disorders, as we understand them; however, they are sufficiently distinctive to be recognized as relatively specific diagnostic entities. Whether or not they are considered ''electro clinical syndromes'' now or in the future is less important than that they be recognized by clinicians who are treating patients. Structural/metabolic epilepsies: The next group includes epilepsies secondary to specific structural or metabolic lesions or conditions but which do not, given our current understanding, fit a specific electro clinical pattern, although that may change in the future. Therefore, these entities represent a lower level of specificity than the two previous groups. Epilepsies of unknown cause: Those epilepsies, which in the past were termed ''cryptogenic,'' will now be referred to as being of ''unknown'' cause.[38]

Rationalising the Use of Antiepileptic Drugs (AEDs)

Most people can have their epilepsy controlled by one or, at the most, two AEDs. If combination therapy is required, a second AED should be chosen which complements the first drug and attention should be paid to each one's influence on the other's metabolism.

Monotherapy with the most appropriate AED should be pursued as far as possible before changing to or adding other agents.

Care should be taken to find the minimum effective dose for any AED and to cease drugs which are clearly non-efficacious.

A routine clinical review should be scheduled every three months for people using AEDs. A careful history and examination is more reliable in determining effectiveness and adverse

effects of treatment than drug serum levels in most situations. The exception is phenytoin, which can reach toxic levels with small dose increases. Monitor for adverse effects, which might indicate the need for dose reduction.[39]

Possible adverse effects:

- Ataxia
- Dysarthria
- Drowsiness
- Nausea and vomiting
- Skin rash
- Decline in cognition
- Agitation
- Other disturbed behaviours
- Gingival hypertrophy (overgrowth of gums) occurs in approximately 30% of people who use phenytoin.
- Rare cases of fatal liver failure have been linked to valproate.
- Vigabatrin and clonazepam may both stimulate aggression in people with developmental disabilities.
- Many people who have developmental disabilities find it difficult to co-operate with sampling for blood tests.

  If a person has been seizure-free for 2-3 years at a constant level of AED, a very slow process of withdrawal of treatment may be undertaken. The process is more likely to be successful if the EEG is normal, although this is by no means invariable and the decision to withdraw medication should be based on clinical judgement rather than the absence of EEG abnormalities.[36]

First aid for convulsive epileptic seizures

- Stay calm.
- Note the time.
- Prevent others from crowding round.

- Put something soft under the person's head like a jacket to prevent injury.
- Only move if they are in a dangerous place, such as in the road or at the top of stairs. Move things away from them if there is a risk of injury.
- Do not attempt to restrain the convulsive movements. Allow the seizure to take its course.
- Do not put anything in the person's mouth. There is no danger of swallowing the tongue and teeth can easily be broken.[17]

2.9 DIAGNOSIS METHODS:

No test can say for certain that you do or do not have epilepsy. But when the information from the tests is added to the description of what happens during your seizures, this builds up a clearer picture of what happened.

This can help with the diagnosis and when choosing treatment.

2.9.1 Electroencephalogram (EEG):

An EEG is used to record the electrical activity of the brain by picking up the electrical signals from the brain cells. These signals are picked up by electrodes on the head and are recorded on paper or on a computer.[40]

The recording shows how the brain is working. Like an ECG, the electrodes only record electrical activity – they do not give out electrical signals and they do not hurt. Before the test, the technician measures your head to work out where to place the electrodes. Each electrode is held in place using a sticky paste. Once the electrodes are attached they are connected to the recording machine. The test lasts about 30 minutes and you will probably be sitting or lying down.[41]

An EEG gives information about the electrical activity of the brain during the time the test is happening. When someone has an epileptic seizure their brain activity changes. This change, known as epileptic form brain activity, can sometimes be seen on an EEG recording. Some people can have epileptic form brain activity even when they do not appear to be having a seizure, so an EEG can be particularly useful for them. Epileptic form activity can sometimes be provoked (brought on) by deep breathing. The test may include deep breathing to see if epileptic form activity can be provoked and recorded.[42]

Flashing lights during the EEG

Some people with epilepsy have seizures that are started, or 'triggered', by flashing lights. This is called photosensitive epilepsy and it affects up to 5% (1 in 20) of people with epilepsy. An EEG will usually include testing for photosensitive epilepsy. This involves looking at a light which will flash at different speeds. If this causes any changes in your brain activity the technician can stop the flashing light before a seizure develops.[43]

2.9.2 Magnetic Resonance Imaging (MRI) scans

An MRI scan looks at the structure of the brain and may help to find the cause of your epilepsy. During the scan detailed pictures are produced using strong magnetic fields. Because of the magnetic fields, metal objects in or near the machine can affect, or be affected by, the machine.

Before having an MRI scan you will need to remove any metal objects such as jewellery, hearing aids, coins or keys. If you have a heart pacemaker or any surgical implant that contains metal you may not be able to have an MRI scan. The scanner makes a loud knocking noise, so before it starts you will be given earplugs to wear. You will also be given a buzzer to hold so you can let the technician know if you are uncomfortable or feeling unwell during the scan.

The technician is usually on the other side of a window in another room during the scan, but an intercom means you can talk to them. There is also a mirror inside the scanner so you can see the technician during the scan. You may be able to have someone in the room with you during the scan.[44]

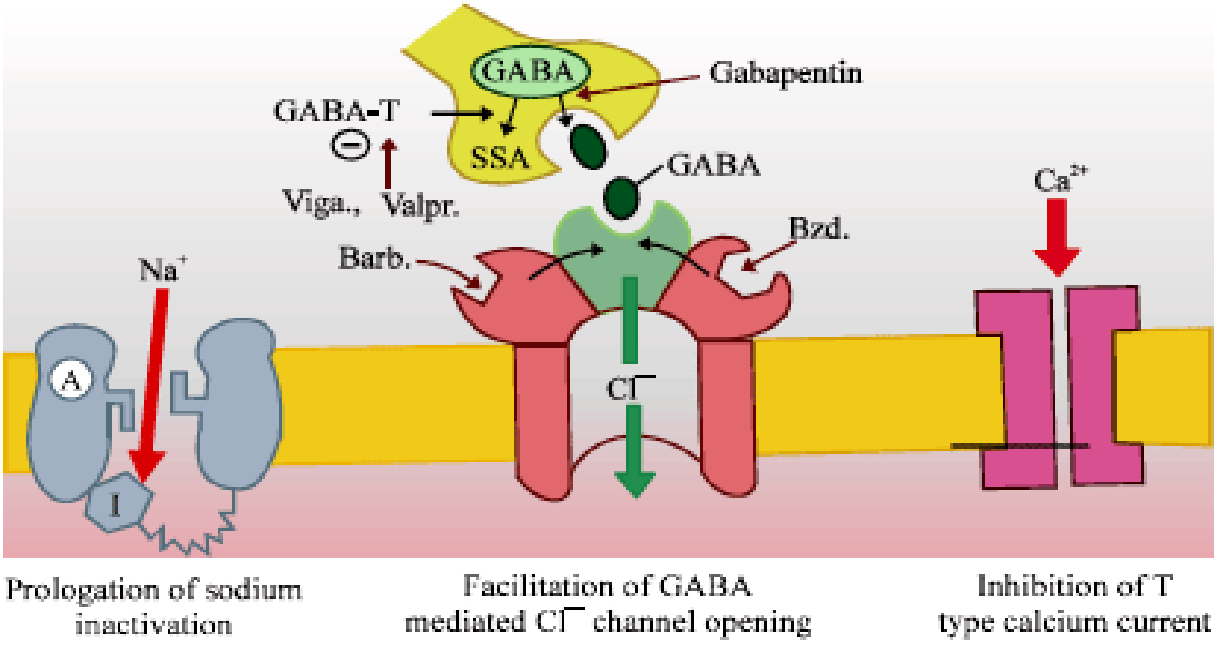
2.9.3 Computerized Axial Tomography (CT or CAT) scan

Some people may have a CT scan if they are not able to have an MRI scan. For example, if they have a heart pacemaker, if they might need to have an anaesthetic to have an MRI, or if information is needed quickly about what might be causing their seizures.

CT scans use X-rays to take images of the brain. (CT scans are not suitable if you are pregnant because the X-rays could affect an unborn baby). Images from a CT scan are less detailed thanthose from MRI scans. During a CT scan you lie on a couch which slides into the scanner. Unlike MRI scanners, CT scanners do not make a loud noise.

2.10 ROLE OF GABA:

Figure 7:



g-Amino butyric acid (GABA), the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is perturbed, seizures may ensue.[45] GABA is formed within GABAergic axon terminals and released into the synapse, where it acts at one of two types of receptor: $GABA_A$, which controls chloride entry into the cell, and $GABA_B$, which increases potassium conductance, decreases calcium entry, and inhibits the presynaptic release of other transmitters. $GABA_A$-receptor binding influences the early portion of the GABA mediated inhibitory postsynaptic potential, whereas $GABA_B$ binding influences the late portion. GABA is rapidly removed by uptake into both glia and presynaptic nerve terminals and then catabolized by GABA transaminase. Experimental and clinical study evidence indicates that GABA has an important role in the mechanism and treatment of epilepsy:

(a) Abnormalities of GABAergic function have been observed in genetic and acquired animal models of epilepsy;

(b) Reductions of GABA-mediated inhibition, activity of glutamate decarboxylase, binding to GABAA and benzodiazepine sites, GABA in cerebrospinal fluid and brain tissue, and

GABA detected during microdialysis studies have been reported in studies of human epileptic brain tissue;

(c) GABA agonists suppress seizures, and GABA antagonists produce seizures;

(d) Drugs that inhibit GABA synthesis cause seizures; and

(e) Benzodiazepines and barbiturates work by enhancing GABA-mediated inhibition.

Finally, drugs that increase synaptic GABA are potent anticonvulsants. Two recently developed antiepileptic drugs (AEDs), vigabatrin (VGB) and tiagabine (TGB) are examples of such agents. However, their mechanisms of action are quite different (VGB is an irreversible suicide inhibitor of GABA transaminase, whereas TGB blocks GABA reuptake into neurons and glia)

Epileptic seizures are generally recognized as a consequence of excitation /inhibition (E/I) imbalance in involved neuronal networks.[46] Restoring appropriate E/I balance by cell-specific modulation at proper sites in the epileptic circuitry is crucial for intervention in seizures. The function of inhibitory neurons has attracted increasing attention due to their essential role in the regulation of neuronal activity and network synchronization, as well as the coordination of animal behaviors. [47, 48, 49]

$GABA_A$ receptors, which mediate the fast synaptic inhibitory action of GABA, are also targets for several medications used in treating seizures, including benzodiazepines or barbiturates[50]. Nineteen genes have been identified coding for distinct subunits, which assemble into a functional $GABA_A$ receptor composed of a combination of five of these subunits. This heterogeneity is reflected in the multitude of structurally distinct $GABA_A$ receptor subtypes expressed in various brain regions.

GABAergic synaptic neurotransmission:

Epileptic seizures are manifestations of synchronized waves of uncontrolled but transient electrical activity in populations of neurons in the brain. Neurons communicate with each other at specialized contact points termed synapses by releasing neurotransmitters that bind to receptors located on the surface of the receiving neuron. Depending on the neurotransmitter, the

type of receptor, and the prevailing ionic gradients, the receiving neuron is either excited or inhibited. In the healthy brain, a delicate balance between excitation and inhibition is maintained that ensures the efficient functioning of neuronal networks. Disease processes that tip the scales in favor of excitation result in agitation or even seizures, while excessive inhibition of large numbers of neurons results in sedation. Accordingly, many antiepileptic drugs act by promoting inhibition in the brain.

2.11 OTHER TREATMENTS

Surgery

If treatment with medications does not work, some individuals with epilepsy may be candidates for brain surgery. To be eligible for the surgery the person must have seizures coming from one area of the brain that can be removed without causing damage to the person's ability to function. If a defect in the brain (such as a scar) can be identified as the cause of the seizures and is confined to a small area, surgically removing that area can eliminate seizures in up to 80% of people, or at least reduce the severity and frequency of seizures.

Vagus Nerve Stimulator

The Vagus Nerve Stimulator is a device that is implanted in a person. Electrical stimulation of the vagus nerve can reduce the number of a certain type of seizures by more than one half in some people. This treatment is used when seizures continue despite use of AEDs and when surgery is not a possibility.

The vagus nerve is thought to have indirect connections to areas of the brain often involved in causing seizures. A device that looks like a heart pacemaker is implanted under the left collarbone and is connected to the vagus nerve in the neck with a wire that runs under the skin. The device causes a small bulge under the skin. The operation to implant the device is done on an outpatient basis and takes about 1 to 2 hours. When people sense that a seizure is about to begin, they can activate the magnet mode of their device to deliver an on-demand dose of stimulation. Otherwise the device is programmed to deliver intermittent stimulation to the vagus nerve. Vagus nerve stimulation is used in addition to AEDs. Side effects of this device include hoarseness, cough, and deepening of the voice when the nerve is stimulated.

Ketogenic Diet

The ketogenic diet, which is very high in fats and low in carbohydrates, was first developed almost 80 years ago. It makes the body burn fat for energy instead of glucose. It has a success rate of 75%, stopping seizures in 50% of individuals and further reducing seizures in 25% of cases. It is a strict diet, and takes a strong commitment from the whole family. The ketogenic diet is not a do it yourself diet. It is a serious form of treatment that, like other therapies for epilepsy, has some side effects that have to be monitored. More research is being done to learn about the underlying reasons for the diet's positive effect.

2.12 ANIMAL MODELS IN EPILEPSY:

Animal models of epilepsy provide information about the pathogenesis of epileptic disorder and for studying the efficacy of potential therapies and their mechanism of action.

Kainic acid model:

Kainic acid was one of the first compounds used to model TLE in rodents[45]. It is an L-glutamate analogue, the systemic or intra-cerebral administration of which causes neuronal depolarization and seizures, preferentially targeting the hippocampus[56].

Pilocarpine model:

Pilocarpine is a muscarinic acetylcholine receptor agonist. Systemic or intracerebral injection of pilocarpine causes seizures that build up into a limbic SE[47, 48]. Structural damages and subsequent development of spontaneous recurrent seizures resemble those of human complex partial seizures[59].

Kindling model:

Kindling is the most studied model of electrical stimulation. Kindling refers to a seizure-induced plasticity phenomenon that occurs when repeated AD induction by electrical stimulation in a specific brain region evokes a progressive enhancement of seizure susceptibility. Ultimately, it culminates in emergence of spontaneous seizures and the establishment of a permanent epileptic state.[60]

Hyperthermic seizure:

Febrile seizures are frequent in early life, with a prevalence of 2%–5% in children younger than 5 years[61] (defined as seizures longer than 15 minutes with focal onset and possible recurrence within 24 hours). Animal models of febrile seizure were developed to investigate whether febrile seizures per se induce neuronal damage leading to epileptogenesis, and which mechanisms generate febrile seizures[62].

Audiogenic model:

Audiogenic seizures (AS) are generalized seizures provoked by high-intensity acoustic stimulation. Activation of auditory pathways is crucial for AS development, and the inferior colliculus in the auditory midbrain plays a key role in audiogenic seizure initiation, although other structures participate in AS progression[63, 64].

Strychnine (STR) induced convulsions:

The mice were randomly divided into groups as described above. The induction of STR induced convulsion was carried out according to previously described method[55]. Immediately after STR administration mice were observed for next 60 min for following symptoms:

1. Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
2. Incidence (number of mice showing convulsions); mortality

Picrotoxin (PTX) induced convulsions:

The mice were randomly divided into groups as described above. The induction of PTX induced convulsion was carried out according to previously described method[56]. Immediately after PTX administration mice were observed for next 30 min for following symptoms:

1. Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
2. Incidence (number of mice showing convulsions); mortality

Pentylenetetrazole (PTZ) induced convulsions:

The induction of PTZ induced convulsion was carried out according to previously described method[57]. Immediately after PTZ administration mice were observed for next 30 min for following symptoms:

1. Onset of convulsion, duration of clonic convulsion, duration of tonic convulsion
2. Incidence (number of mice showing convulsions); mortality

Table 1: Common methods used to induce convulsion in animal models[68]

Animal models	Methods to induce convulsion	Types of seizures
Invivo model	**Electrical stimulation:**	
	Maximal electroshock(MES)	Generalised tonic-clonic seizures
	Kindling	Chronic partial seizures
	Chemoconvulsants:	Myoclonic and absence seizures
	Pentylenetetrazole (PTZ)	Acute simple partial seizures
	Strychnine	Acute simple partial seizures
	Picrotoxin	Clonic-tonic seizures
	Isoniazid	Status epilepticus
	Lithium pilocarpine	Clonic seizures
	Yohimbine	Acute simple partial seizures
	Bicuculline	Clonic-tonic seizures
	4-aminopyridine	Status epilepticus
	N-methyl d- aspartate	Generalised tonic-clonic and
	Penicillin	

		Absence Seizures
Invitro model	Hippocampal slices $GABA_A$ receptor binding Assay	Complex partial seizures
Genetic model	Photosensitive baboons Audiogenic seizures mice Totterer mice and seizures -prone mouse Strains Genetically epilepsy- prone rats	Generalised tonic- clonic seizures

TABLE 2: ANTI-EPILEPTIC DRUG CLASSIFICATION

S. NO.	CLASSIFICATION	DRUG NAME
1	Hydantoins	Phenytoin, mephenytoin
2	Barbiturates	Phenobarbitone, mephobarbitone
3	Deoxybarbiturate	Primidone
4	Iminostilbene	Carbamazepine
5	Succinimide	Ethosuximide
6	GABA transaminase inhibitors	Valproic acid, vigabatrin
7	Benzodiazepines	Diazepam, clonazepam, lorazepam, clorazepate
	NEWER AGENST	

8	GABA analogues	Gabapentin, vigabatrin, tiagabine
9	Others	Lamotrigine, levetiracetam, felbamate, topiramate, zonisamide, lacosamide, rufinamide

TABLE 3: DRUG PROFILE FOR ANTI-EPILEPTIC DRUGS

S. NO.	DRUG NAME	MOA	ADVERSE EFFECT	USES
1	Phenytoin	Blockade of voltage dependent Na^+ channels	• Gingival hyperplasia • Hypersensitivity • Megaloblastic anaemia • Teratogenicity (foetal hyndatoin syndrome)	➢ Tonic-clonic seizure ➢ Trigeminal neuralgia ➢ Cardiac arrhythmias
2	Phenobarbitone	Facilitated GABA mediated opening of Cl^- ion channels	• Sedation • Nystagmus • Megaloblastic anaemia • Osteomalacia • Skin rashes	➢ Generalized tonic-clonic seizures ➢ Partial seizure
3	Primidone	Same as phenytoin	• Same as phenobarbitone	➢ Generalized tonic-clonic seizures ➢ Partial seizure
4	Carbamazepine	Blocks sodium channels	• Antidiuretic effect	➢ Trigeminal neuralgia ➢ Glossopharyngeal neuralgia
5	Ethosuximide	Reduces low threshold calcium currents	• Epigastric pain • Anorexia • Euphoria • Lethargy • Thrombocytopenia	➢ Absence Seizure

6	Valproic acid	Increase the level of GABA Blocks sodium channel Decrease low threshold Ca^{++}	• Hepatotoxicity • Teratogenecity Cause Neural Tube Defects • Idiosyncratic Thrombocytopenia • Alopecia • Ataxia • Rashes	➢ Partial And Generalized Seizure ➢ Absence Seizure ➢ Mood stabilizer
7	Diazepam	Potentiate the GABA action	• Constipation • Blurred Vision • Slurred Speech • Hypotension • Hangover	➢ Febrile convulsion
8	Gabapentin	Enhance release of GABA	• Dizziness • Ataxia • Tremor • Rhinitis	➢ Partial seizure
9	Vigabatrin	Irreversible inhibition of GABA transaminase enzyme	• Depression	➢ Refractory epilepsy
10	Tiagabine	Depression of GABA transporter GAT-1	• Nervousness • Amnesia • Asthenia • Sedation	➢ Partial seizure
11	Topiramate	Blocks Na^+ channel Blocks AMPA receptor Enhance $GABA_A$ current	• Fatigue • Drowsiness • Dizziness • Formation of renal calculi • Dysguisia • Cognitive dysfunction	➢ Partial, generalized seizure ➢ Absence seizure

Table 4: Correlation between mechanisms of epileptogenesis and mechanisms of action of AEDs

	Mechanisms of epileptogenesis	Mechanisms of actions of AEDs
GABA	• Reduced GABA in microgyric cortex • Reduced benzodiazepine receptor binding in medial thalamic nucleus (*mesial temporal lobe epilepsy*) • Reduced benzodiazepine receptor density in CA1 region (*hippocampal sclerosis*) • Reduced GABA levels and GAD activity (*epileptic foci*) • Auto-antibodies to GAD (*Stiff-man syndrome*)	• Increased functional pool of GABA (*vigabatrin, tiagabine*) • Enhanced GABA-ergic inhibition (*benzodiazepines*) • GABA agonistic effects (*progabide*) • (Weaker) GABA-ergic properties (*phenobarbital, gabapentin, topiramate, valproate, zonisamide*)
Glutamate	• Upregulation of hippocampal ionotropic glutamate receptors (*temporal lobe epilepsy*) • Anti-gluR3 antibodies (*Rasmussen encephalitis*) • Increased plasma glutamate levels (*absence seizures*)	• Inhibition of glutamate release (*lamotrigine*) • Block of glycine site at NMDA receptor (*felbamate*)
Sodium channel	• Mutation voltage-gated Na+ channel (*generalized epilepsy with febrile seizures*)	• Reduction of voltage-gated Na+ currents (*carbamazepine, felbamate, lamotrigine, oxcarbazepine, phenytoin, topiramate, valproate, zonisamide*)
Potassium channel **Calcium channel**	• Mutation voltage-gated K+ channel (*benign familial neonatal convulsions*) • Reduced ACh-mediated Ca flux _ (*nocturnal frontal lobe epilepsy*)	• Reduction of T-type Ca++ currents (*ethosuximide, valproate*)

PLANT PROFILE

DESCRIPTION

Plant name : *Cassia alata* Linn.

Family : Caesalpiniaceae

Scientific Classification

Kingdom : Plantae

Order : Fabales

Family : Fabaceae

Subfamily : Caesalpinioideae

Tribe : Cassieae

Subtribe : Cassiinae

Genus : Senna

Species : S. alata

Bionomial name : Senna alata

Synonyms : *Cassia alata* Linn.

Vernacular names:

Tamil : Semaiagathi, Vandugolli

English : Ringworm shrub, winged senna

Sanskrit : Dadrughna, Dvipagsti[69]

Cassia alata Linn. is a large shrub with thick downy branches, found wild almost throughout India. Leaflets are 8-12 pairs, lower leaflet oblong-elliptic; upper ones broadly obovate[70]. It is also known as Ringworm shrub and winged senna in English. The leaves of the plant are used as purgative, expectorant astringent, vermicide and to treat all skin diseases[71]. Extracts of *Cassia alata* leaves have been reported to possess analgesic, anti-bacterial, anti-inflammatory, fungicidal, hypoglycaemic, laxative, and oxytocic and wound healing activity[72].

Figure 8

Figure 9

(7) *Cassia alata* Linn. whole plant (8) *Cassia alata* Linn. flower

It is a pan tropical, ornamental shrub. Its seeds are reported to be alternative of legumes due to high proteins and carbohydrates[73].

In Ayurveda, these plants are reported to be useful in jaundice, and in other traditional system of medicine are highly valued for treatment of various ailments[74].

Cassia alata Linn. leaf obtain from Neigeria having some phytochemical groups in their leaves and roots that has been reported to be useful in treating constipation, food poisoning, burns, wounds, and ringworm as well as exzema[75].

4. LITERATURE REVIEW

In Vitro Anthelmintic Activity of Leaf Ethanolic Extract of *Cassia alata* and Typha Angustifolia

The ethanolic extract of *Cassia alata* and Typha angustifolia have shown invitro Anthelmintic activity. Tannin like polyphenolic compounds produces that anthelmintic activity[76].

Antimicrobial Activity of *Cassia alata* and Phyllanthus amarus

The *Cassia alata* showed antimicrobial activity. Antifungal activity of aqueous extract of *Cassia alata* was found against Candida albicans by using zone of inhibition method[77].

Antibacterial and antioxidative compounds from *Cassia alata* Linn.

The *Cassia alata* showed weak antibacterial activity against Gram-positive bacteria. Emodin isolated from the *Cassia alata*, which is the main constituent possess antibacterial activity[78].

Antipyretic activity of aqueous and ethanolic extracts of *Cassia alata* Linn Leaf.

The aqueous and ethanolic extracts of *Cassia alata* possess antipyretic activity. Ethanolic extract having higher antipyretic activity than water extract[79].

In vitro antimalarial activity of 11 terpenes isolated from *cassia alata* and *ocimum gratissimum* leaves. screening of their binding affinity with haemin

The *Cassia alata* having antimalarial activities by the presence of isolated terpene as 4 terpenes from *Cassia alata*. Terpenes from *Cassia alata* and *Ocimum gratissimum* are very active compounds in malaria treatment[80].

Effects of *Cassia alata* Root Extract on Smooth Muscle Activity

The root extracts of *Cassia alata* showed Smooth muscle activity like oxytocin and prostaglandin in the enhancement of labour during maternal delivery. Used as abortifacient and laxative drugs especially in life threatening situation[81].

Antimicrobial activity of *Cassia alata*

The methanolic extracts of leaves have shown antimicrobial activity and tested in bacteria and fungi. The of *Cassia alata* have very strong activity against bacteria and fungi due to presence alkaloidal salts and base. So the plant (flower and leaf) can be useful for the treatment of fungal skin diseases[82].

Acetylcholinesterase inhibition by some promising Brazilian medicinal plant

The plant extract showed acetylcholinesterase inhibition properties. The *Cassia alata* species possess activity similar to the galanthamine. So it can be useful for the treatment of Alzheimer disease along with antioxidant activity and anti-inflammatory activity[83].

Antiviral activity of *Cassia alata* Linn. extracts against cardiac coxsackievirus B3 infections in vitro and in vivo

The aqueous extract of *Cassia alata* showed potential antiviral agent for the treatment of myocarditis. And the aqueous extract significantly reduced the morbidity, mortality, virus titers, necrosis and mononuclear cell infiltration of heart tissues. Also the extract shows the ability to maintain levels of LDH, AST, and CK enzymes at normal level in the treated infected mice compared with those untreated infected mice[84].

Weight-lowering effects of *Cassia alata*, Cassia fistula and Caesalpinia pulcherrima leaf extracts

The *Cassia alata* and *Cassia fistula* can significantly and effectively reduced the body weight and weight of parametrial fat in mice possibly due to their tannin contents which inhibit the activity of lipases thereby lowering their body fat content[85].

Antibacterial activity of leaf extract of *Cassia alata* separated by soxhlet extraction method

The leaves of *Cassia alata* showed antibacterial activity against the bacterial strains Pseudomonas aeruginosa which was compared with standard drugs[86].

Antioxidant and Anti-Inflammatory Activities of Extracts from *Cassia alata*, *Eleusine indica*, *Eremomas taxspeciosa*, *Carica papaya* and *Polyscias fulva* Medicinal Plants Collected in Cameroon

The *Cassia alata* having Antioxidant and Anti-Inflammatory activities. The extracts from leaves of *Cassia alata* have better antioxidant activities than Carica papaya or Eleusine Indica. The presence of Chrysarobin, tannin, Kaempferol, Isochrysophanol, Chrysophanol glycoside Chrysarobin explained Antioxidant and Anti-Inflammatory[87].

Inhibitors of platelet aggregations

The isolated adenine from *Cassia alata* Linn. posses strong inhibitory effects on platelet aggregation induced by collagen. Here the adenine was isolated by HPLC using a tryacontylsilyl silica (C_{13})[88].

Invitro antifungal activity of *Cassia alata* Linn. flower extract

The aqueous extract of *Cassia alata* showed inhibition of growth of aflatoxin produced from plant and human pathogenic fungi[89].

Effect of Cassia alata (L) Roxb (Fabaceae) Leaf Extracts on alpha-Amylase, alpha-Glucosidase and Postprandial Hyperglycemia in Rats

The acetone and hexane extracts of *Cassia alata* showed anti-diabetic activity by inhibition of α- amylase and α-glucosidase. The mode of inhibiting both enzymes is competitive and uncompetitive inhibition, respectively. The hexane extract also reduces sucrose-induced postprandial hyperglycaemia in rats. So *Cassia alata* leaf extracts can inhibit α-amylase as well as α- glucosidase and reduced postprandial hyperglycaemia in diabetes induced rats.[90]

Anti-inflammatory Activity of Heat-treated *Cassia alata* Leaf Extract and Its Flavonoid Glycoside

Cassia alata leaves showed various anti-inflammatory activities. The inhibitory effects on the histamine release induced by Concanavalin A were notable in the extracts of heat-treated[81].

5. SCOPE AND PLAN OF WORK

Scope:

According to the WHO, there are over 50 million suffers in the world today of which 80% live in the developing countries. An estimated 3.2 million new cases occur each year globally, with atleast 55% of the cases begin in childhood. Epilepsy responds to treatment about 75% of the time, some cases show poor treatment due to inadequate medical supply and proper treatment. Epilepsy increases a person's risk of premature death by about two to three times. It is the most common serious brain disorder worldwide with no boundaries.

Management of epilepsy is a global problem and successful treatment is very essential for preventing or at least delaying the onset of long-term complications. Remedies to treat such different types of seizure are available in nature in the form of herbal medicines or drugs with very minimal adverse effects when compared to the available synthetic drugs. Such herbal drugs as therapeutic agents are a boon when compared to the severe adverse effects of the allopathic medical practice for epileptic seizure, though the quest for a complete and permanent cure for the disease is being pursued relentlessly by eluding physicians and researchers.

Therefore herbal medicines have been used for the treatment of various disease because of their fewer adverse effects that conventional medicine. It is believed that the traditional medicines used for the treatment of epilepsy and also in progression of complications of the disease.

Main objective of the proposed work is to evaluate the beneficial effects of *Cassia alata* Linn. for its anti-epileptic activity by using MES and INH model.

PLAN OF WORK

6. PLAN OF WORK

1. Collection and Authentification of *Cassia alata* Linn.
2. Preparation of ethanolic extract of *Cassia alata* Linn. (EECA)
3. Experimental Animals
4. Preliminary Phytochemical screening
5. Acute toxicity studies (OECD 423guidelines)
6. Effect of EECA on MES induced epilepsy
7. Effect of EECA on INH induced epilepsy
8. Effect of EECA on GABA level in animal
9. Histopathology
10. Statistical analysis by one way ANOVA followed by Dunnet's test

MATERIALS AND METHOD:

6.1 Collection and Authentification:

The leaves of *Cassia alata* Linn. were collected from local source, Tamil Nadu in March. The plant material was identified and authenticated by Dr. D. Aravind, M.D. (S), M.Sc., Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai– 600047, Tamilnadu. Certificate number NISMB2132016. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai- 600097

6.2 Preparation of ethanolic extract of *Cassia alata* Linn. (EECA)

The leaves of *Cassia alata* Linn. was collected and ground into coarse powder. The powdered leaves were extracted with ethanol in Soxhlet's apparatus.

6.2.1 Method of Extraction:

The powdered leaves were extracted with ethanolic solvent at room temperature for 24 hours using soxhlet's apparatus. The extract process was repeated twice. The combined filtrates were then evaporated under reduced pressure to give a viscous mass, which gave a greenish golden coloured residue. The extract was stored at 0-4°C.

6.2.2. Percentage yield:

The percentage yield of hydro alcoholic extract was 2.77% w/w and it was preserved in refrigeration for further use.

6.3. Experimental Animals:

Swiss albino mice of weighing 25-30gm were used for this study. The inbred animals were procured from the animal house of C.L. Baid Metha College of Pharmacy, Thorapakkam, Chennai- 97. They were housed five per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C with 12 hr light/dark cycle. The animals were acclimatized to laboratory conditions for one week provided with standard pellet chow and water *ad libitum*. Ethical committee approval was obtained from IAEC of CPCSEA. (IAEC/XLVII/01/CLBMCP/2015 dated: 20/11/2015)

6.4 Phytochemical analysis: [92]

The *Cassia alata* Linn. was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

I. Test for alkaloid:

The extract was treated with dilute hydrochloric acid and filtered. The filtrate was used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d**) Wagner's test (Iodine-Potassium Iodide Solution)**

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

II. Test for Carbohydrates

a) Molisch's test:

The extract was treated with 3ml of alpha-napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test ($CuSO_4.7H_2O$+KOH+Potassium Tartartes):

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + $CuSO_4.7H_2O$)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non- reducing sugars.

III. Test for steroids

a) Liberamannburchard test:

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids

IV. Test for proteins

a) Biuret's test:

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test:

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

V. Test for Tannin's

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

VI. Test for Phenols

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

VII. Test for Flavonoid

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavonoids.

VIII. Test for Gums and Mucilage

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

IX. Test for Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the yjunction of two liquids indicates the presence of glycosides.

X. Test for Saponins

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

XI. Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

XII. Test for sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

6.5. Acute toxicity studies[93]

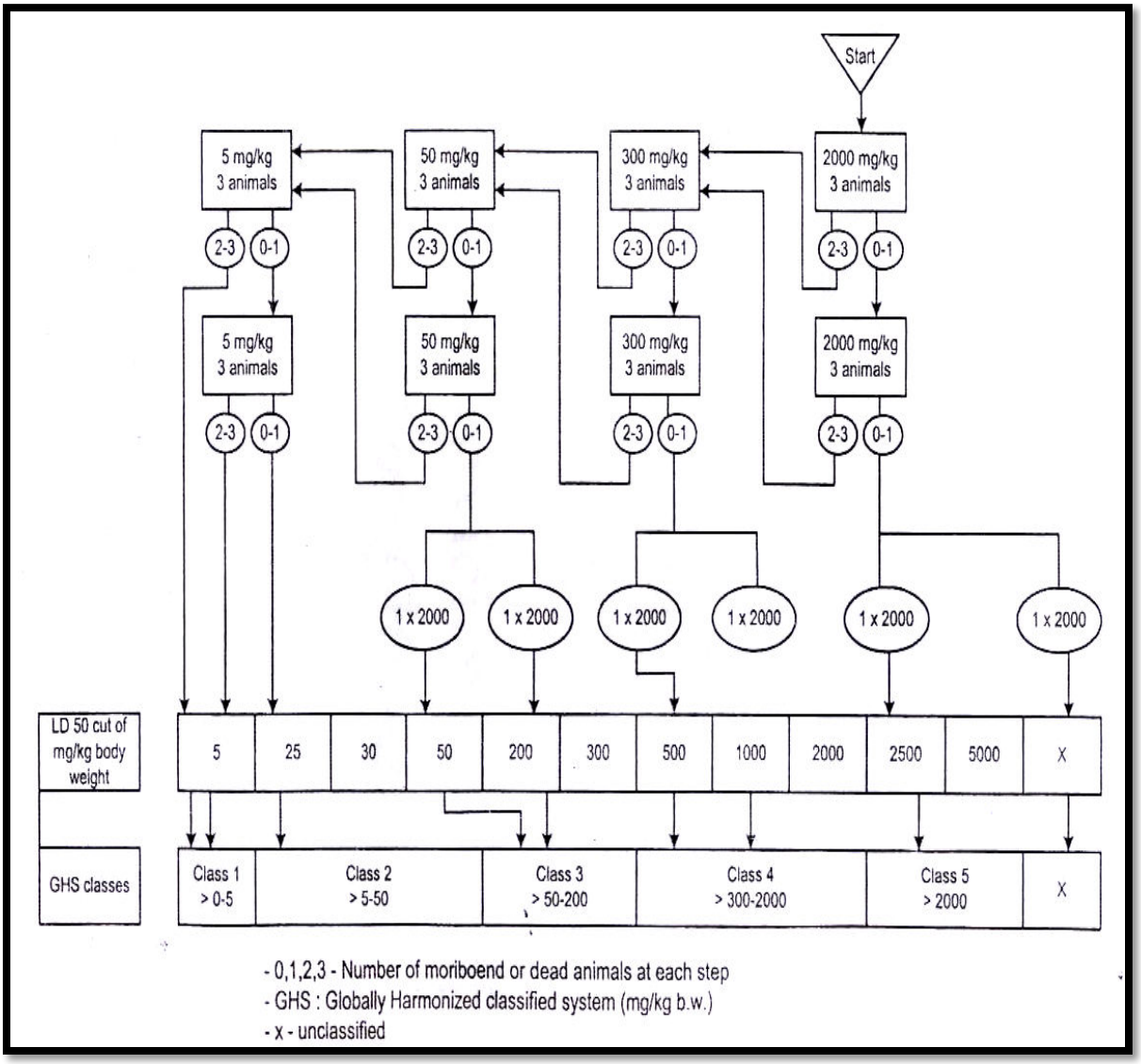
The procedure was followed by using OECD guidelines (Organization for Economic Co-Co-operation and Development) 423(acute toxic class method). The acute toxicity study is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances. This procedure results in the use of a specified number of animals while allowing for acceptable data-based scientific conclusion. The method used defined doses (2000mg/kg body weight) and results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for classification of chemical which cause acute toxicity.

Procedure:

Swiss albino mice weighed 25-30gms were used for the study. The starting dose level of EECA was 2000mg/kg body weight p.o Most of the crude extracts possess LD_{50} value more than 2000 mg/kg, p.o so starting dose used was 2000mg/g p.o Food was withheld for a further 3-4 hrs.After administration (p.o) of drugs and observed for the signs of toxicity.

Body weights of mice before and after administration were observed for morbidity and mortality. Any changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behaviour pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted.

Figure 10: Flowchart of acute toxicity studies



Experimental Design

Anti-epileptic activity

Method- I

6.6. Maximal electroshock seizures (MES) Convulsions[94, 95]

Procedure:

The animals were divided into 5 groups each constituting 6 mice. Group I were normal mice, Group II were negative control. Group III animals were treated with phenytoin 25mg/kg b.w/p.o. Group IV were treated with EECA 200mg/kg b.w/ p.o. and Group V were treated with EECA 400mg/kg b w/p.o. for 14 days.

MES model is one of the physical method to evaluate the anti-epileptic activity of drug. Seizures were induced to group II, III, IV and V by using an Electoconvulsiometer. Maximal electroshock seizures were elicited by a 60 HZ alternating current of 50mA intensity for 0.2 sec[96] was applied to the animal using corneal electrode. On 14th day extracts were administered before the induction of seizures. The various stages of epilepsy like Flexion, Extensor, Clonus, Stupor and Recovery were observed. The percentage protection was estimated.

Group I- Normal control

Group II – Negative control MES 60 Hz alternating current of 45mA intensity for 0.2 sec

Group III- Phenytoin 25mg/kg p.o. + MES 60 Hz alternating current of 45mA intensity for 0.2 sec

Group IV-EECA 200mg/kg p.o + MES 60 Hz alternating current of 45mA intensity for 0.2 sec

Group V- EECA 400mg/kg p.o + MES 60 Hz alternating current of 45mA intensity for 0.2 sec

Method- II

6.7. Isoniazid (INH) induced seizures[97, 98]

The animals were divided into 5 groups each constituting 6 mice. Group I were normal mice, Group II were negative control. Group III animals were treated with phenytoin 25mg/kg b.w/p.o. Group IV were treated with EECA 200mg/kg b.w/ p.o. and Group V were treated with EECA 400mg/kg b w/p.o. for 14 days.

INH model is one of the chemically induced method to evaluate anti-epileptic activity of the drug. On 14th day extracts were administered before the induction of seizures. Seizure is induced to groups II, III, IV and V by using intraperitoneal administration of 300 mg /kg of Isoniazid in mice. Status of animal live after 30min, 24 hrs and Percentage protection were calculated.

Group I - Normal control

Group II - Negative control Isoniazid 300mg/kg i.p.

Group III - Standard phenytoin 25mg/kg + Isoniazid 300mg/kg i.p

Group IV - Low dose 200mg/kg p.o + Isoniazid 300mg/kg i.p.

Group V - High dose 400mg/kg p.o + Isoniazid 300mg/kg i.p.

6.8. Determination of the effect of EECA and standard on neurotransmitter concentration in mice brain after induction of epilepsy

Estimation of GABA level in mice brain:

Preparation of standard solutions

1 N HCl was prepared in 80% ethanol which was used to dissolve GABA (γ-amino butyric acid) and glutamate

Preparation of stock solution

Stock solutions of GABA and glutamate were prepared by dissolving 10 mg of the respective amino acid in 10 ml of 0.1 N HCl in 80% ethanol. From this stock solution, working standard solutions of concentration 10ng, 20ng, 40ng, 80ng, 120ng, 160ng, 200ng in 5µl for GABA were prepared in 10 ml volumetric flasks and adjusting the volume with 0.1 N HCl in 80% ethanol.

Preparation of 0.2% ninhydrin solution

200 mg of ninhydrin was dissolved in a small amount of acetone in a 100 ml standard flask. To this, 1 ml of pyridine was added and the volume was made up to 100 ml with acetone

Chromatographic condition

Stationary phase	:	Silica gel GF254;
Mobile phase	:	n-butanol: glacial acetic acid: water (65:15:25 v/v/v);
Saturation time	:	3 hr;
Instrument	:	HPTLC (Camag–version 1.3.4);
Applicator	:	Linomat V;
Scanner	:	Camag TLC scanner III;

Developing chamber	:	Twin trough glass chamber (20×10);
Developing mode	:	Ascending mode (multiple development);
Detection reagent	:	0.2% ninhydrin in acetone;
Scanning wavelength	:	486 nm;
Experimental condition	:	25ºC; Temp/RH: 55–65%.

Calibration curve

Ten µl of different concentration of standard 5µl of GABA and glutamate standard solution were applied in triplicate on a pre-coated HPTLC plate. Spots were dried in a hot air oven at 60–65^{0}C for 1–2 min and the plate was developed in the mobile phase n-butanol: glacial acetic acid: water (65:15:25 v/v/v). When the solvent front reached about 8.0 cm (marked previously), the plates were removed and dried at 60–65^{0}C for 3–4 min in a hot air oven. A second run was performed in a similar way. The plates were then dipped in 0.2% ninhydrin reagent for 1 sec and dried in a hot air oven at 60–65^{0}C for 3–4 min. The spots were scanned at 486 nm and the peak areas were recorded. Calibration curves of GABA and glutamate were prepared by plotting areas v/s concentration.

Calculation

- Brain samples were homogenized each 10mg in 200µl of solvent. Hence 1 mg tissue needs 20µl volume.
- Final results were expressed of ng of protein per mg of tissue.
- Total 10µl of sample volume were taken to measure neurotransmitter quantity. These samples gave peak area as a measure of concentration that was interpolated from standard plot with the help linear regression statistics of graph pad prism 4.01.
- To express the neurotransmitter quantity in ng/mg tissue, final interpolated concentrations from standard plot were multiplied by 2.

6.9 METHODS FOR HISTOPATHOLOGICAL STUDY[99]

The mice from each group were anaesthetized using inhalation of chloroform. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice cold normal saline and fixed in 10% formalin saline.

Paraffin embedded sections were taken 100µm thickness and processed in alcohol-xylene series and stained with Haematoxyli-Eosin dye. The sections were examined microscopically for histopathological changes in the cortex zone.

6.10 STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's ―t test. P values <0.05 (95% confidence limit) was considerd statistically significant, using Software Graph pad Prism 6.01

7. RESULTS

7.1 Preliminary Phytochemical analysis of Ethanolic extract of *Cassia alata* Linn. (EECA)

The result of preliminary phytochemical analysis of Ethanolic extract of *Cassia alata* Linn. showed presence of various phytochemical constituents such as Carbohydrates, phenols, Flavonoids, steroids, alkaloids, glycoside and saponins with absence of terpenes, sterol, protein, tannins, gums and mucilage. **(Table 6)**

7.2 Acute Oral Toxicity Study:

The Acute Oral Toxicity Study was done according to the OECD guidelines 423 (Acute toxic class method). A single administration of 2000 mg/kg b.w /p.o of EECA was administrated to three adult female Swiss albino mice and observed for 7 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. **(Table 7)**

7.3 Effect of EECA on MES induced epilepsy model:

Effect of EECA on Flexion in MES induced epilepsy model:

Flexion in MES induced epilepsy model, group I was significantly decreased when compared with group II, III, IV and V ($p<0.001$).

Group II was significantly increased when compared with group III, IV and V ($p<0.001$).

Group III was significantly increased when compared with Group IV and V ($p<0.001$).

(Table 8, Figure 11)

Effect of EECA on Extensor in MES induced epilepsy model:

Extensor in MES induced epilepsy model, group I was significantly decreased when compared with Group II, III, IV and V ($p<0.001$).

Group II was significantly increased when compared with group III and IV ($p<0.001$).

Group II was significantly decreased when compared with group V ($p<0.001$).

Group III was significantly decreased when compared with group IV and V ($p<0.001$).

(Table 8, Figure 12)

Effect of EECA on Clonus in MES induced epilepsy model:

Clonus in MES induced epilepsy model, group I was significantly decreased when compared with group II, III, IV and V ($p<0.001$).

Group II was significantly increased when compared with group III, IV and V ($p<0.001$).

Group III was significantly decreased when compared with group IV and V ($p<0.001$). **(Table 8, Figure 13)**

Effect of EECA on Stuper in MES induced epilepsy model:

Stuper in MES induced epilepsy model, group I was significantly decreased when compared with group II, III, IV and V ($p<0.001$).

Group II was significantly increased when compared with Group III, IV and V ($p<0.001$).

Group III were significantly decreased when compared with group IV ($p<0.001$).

Group III was significantly increased when compared with group V ($p<0.001$). **(Table 8, Figure 14)**

Effect of EECA on Recovery in MES induced epilepsy model:

Recovery in MES induced epilepsy model, group I was significantly increased when compared with Group II, III, IV and V ($p<0.001$).

Group II was significantly increased when compared with Group III, IV and V ($p<0.001$).

Group III was significantly decreased when compared with Group IV ($p<0.001$).

Group III was significantly increased when compared with Group V ($p<0.001$) **(Table 8, Figure 15)**

Effect of EECA on GABA level in MES induced epilepsy model:

GABA levels in MES induced epilepsy model, group I was significantly increased when compared with Group II, III, IV and V ($p<0.001$).

Group II was significantly decreased when compared with Group III, IV and V ($p<0.001$).

Group III was significantly increased when compared with Group IV and V ($p<0.001$). **(Table 9, Figure 17)**

7.4 Effect of EECA on INH induced epilepsy model:

Effect of EECA on Latency in INH induced epilepsy model:

Group I was significantly increased when compared with Group II, III, IV and V ($p<0.001$).

Group II was significantly increased compared with group III, IV and V ($p<0.001$).

Group III was significantly increased compared with group IV and V ($p<0.001$). **(Table 10, Figure 18)**

Status of animal live in INH model at 30 min interval:

Status of animal live in INH model at 30 min interval, group I was significantly decreased when compared with Group II, III, IV and V.

Group II was significantly decreased when compared with group III, IV and V.

Group III was significantly increased when compared with Group IV and V were. **(Table 10, Figure 19)**

Status of animal live in INH model at 24hrs interval:

Status of animal live in INH model at 24hrs interval, group I was significantly decreased when compared with group II, III, IV and V.

Group II was significantly decreased when compared with group III, IV and V.

Group III was significantly decreased when compared with group IV and V. **(Table 10, Figure 20)**

Percentage protection in INH model:

Percentage protection in INH model, group I was significantly decreased when compared with group II, III, IV and V.

Group II was significantly decreased when compared with group III, IV and V.

Group III was significantly increased when compared with group IV and V. **(Table 10, Figure 21)**

Histopathology: (Figure 22)

Table: 5 HISTOPATHOLOGICAL ANALYSIS OF MICE BRAIN

GROUP	REPORT
CONTROL	Haematoxylin and Eosin stained section shows the normal brain tissue depicted intact cell architecture with normal amount of neurotransmiters.
MES (60 Hz alternating current of 45mA intensity for 0.2 sec)	Haematoxylin and Eosin stained section shows there is less neuron density.
Phenytoin (25mg/kg/p.o) + MES (60 Hz alternating current of 45mA intensity for 0.2 sec)	Haematoxylin and eosin stained section of the brain tissue showed no significant alterations observed in this group and tissues showed a normal picture or brain cells, less proliferation and more neuronal density at hippocampal region
EECA (200mg/kg/p.o) + MES (60 Hz alternating current of 45mA intensity for 0.2 sec)	Haematoxylin and Eosin stained section of the brain tissue showed no pathological damages and cellular architecture are intact with more neuronal density compared to the MES alone treated group.
EECA (400mg/kg/p.o)+ MES (60 Hz alternating current of 45mA intensity for 0.2 sec)	Haematoxylin and Eosin stained section of the brain tissue showed increased neuron density when compared to the EECA (200mg/kg/p.o)

FIGURES AND TABLES

7.1 Preliminary Phytochemical analysis of hydro alcoholic extract of leaves of *Cassia alata* Linn.

Table: 6

S. No.	Phytochemical	Presence or absence
1	Alkaloids	+
2	Carbohydrates	+
3	Steroids	+
4	Proteins	-
5	Tannins	-
6	Phenol	+
7	Flavonoids	+
8	Gums and mucilage	-
9	Glycoside	+
10	Saponins	+
11	Terpene	-
12	Sterols	-

Table: 7 Acute oral toxicity studies of EECA (OECD 423 guideline)

S. No.	Treatment group	Dose	Weight of animal in gm		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	EECA	2g/kg	25	28	No signs of toxicity	Nil	Nil	14 days
2.	EECA	2g/kg	25	25	No signs of toxicity	Nil	Nil	14 days
3.	EECA	2g/kg	30	32	No signs of toxicity	Nil	Nil	14 days

TABLE: 8 EFFECT OF EECA ON MES INDUCED CONVULSIONS

GROUP	% OF PROTECTION	FLEXION (SEC)	EXTENSOR (SEC)	CLONUS (SEC)	STUPOR (SEC)	RECOVERY (SEC)
GROUP I	NIL	0	0	0	0	0
GROUP II	0%	3.167 ±4.401a***	12.00±0.577a***	17.33± 0.88a***	25.00± 2.477a***	127± 4.102a***
GROUP III	100%	2.833±0.307 a***b***	10.50±0.428a***b***	9±2.309a***b***	20.833±1.014a***b***	122±3.130a***b***
GROUP IV	66.6%	2.500±0.428a***b***c***	11.33±0.822a***b*** c***	12.67±1.76a***b*** c***	23.16±1.515a***b*** c***	126±2.017a***b*** c***
GROUP V	83%	1.833±0.307a***b***c***	13.0±0.7303a***b*** c***	11.33±0.803a***b*** c***	19.66±0.919a***b*** c***	117.00 ±2.633a***b*** c***

Values are expressed as mean ± SEM of 6 animals.
Comparisons were made between the following:
a - Group I vs. II, III, IV and V, b - Group II vs. III, IV and V, c- Group III vs. IV and V.
Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.

Figure :11

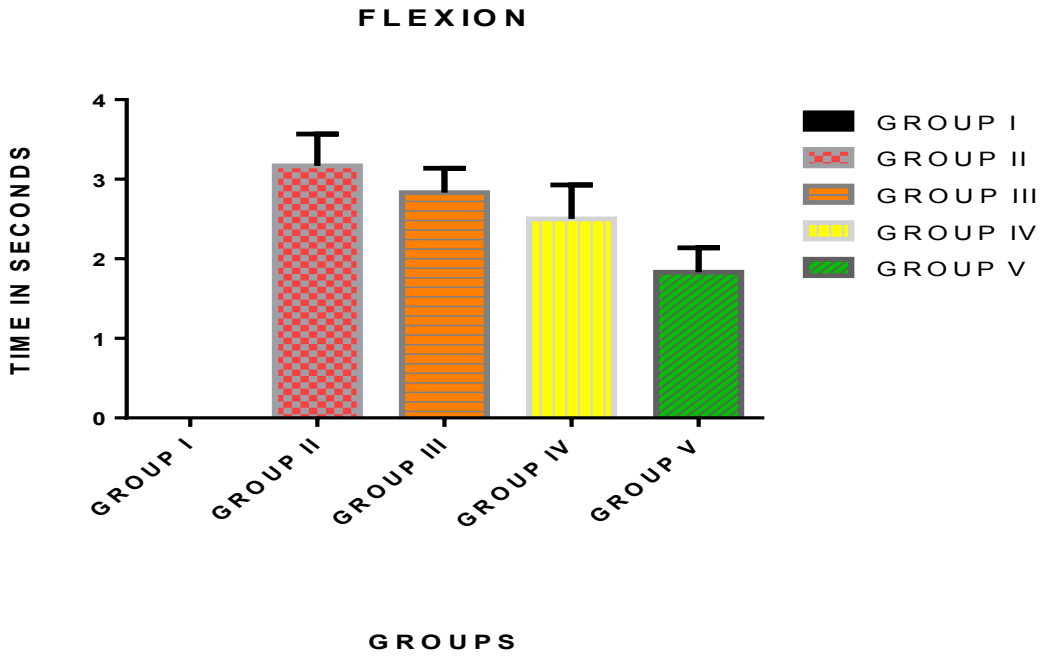


Figure 12:

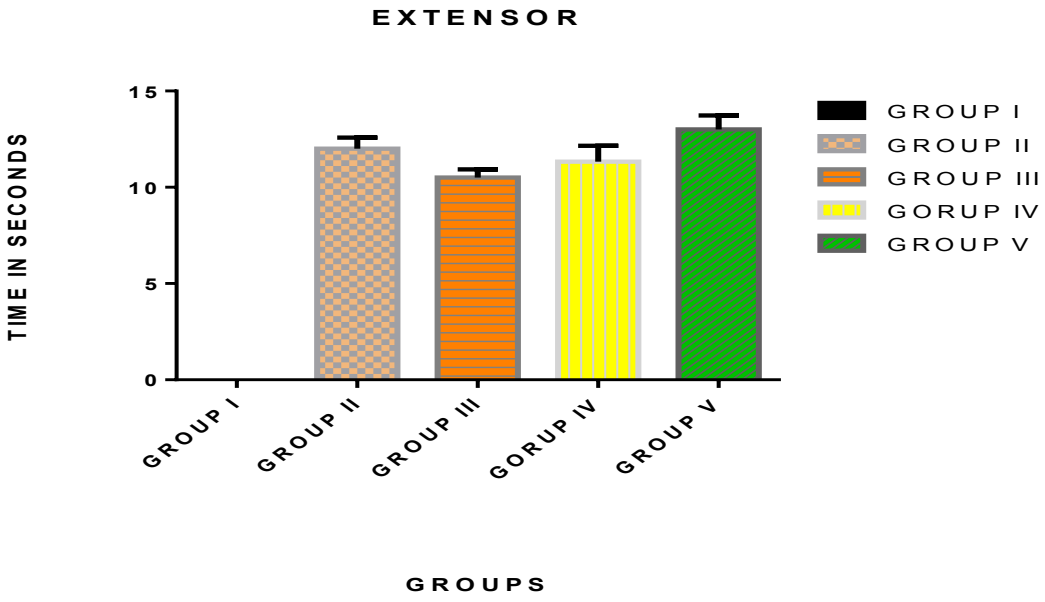


Figure 13:

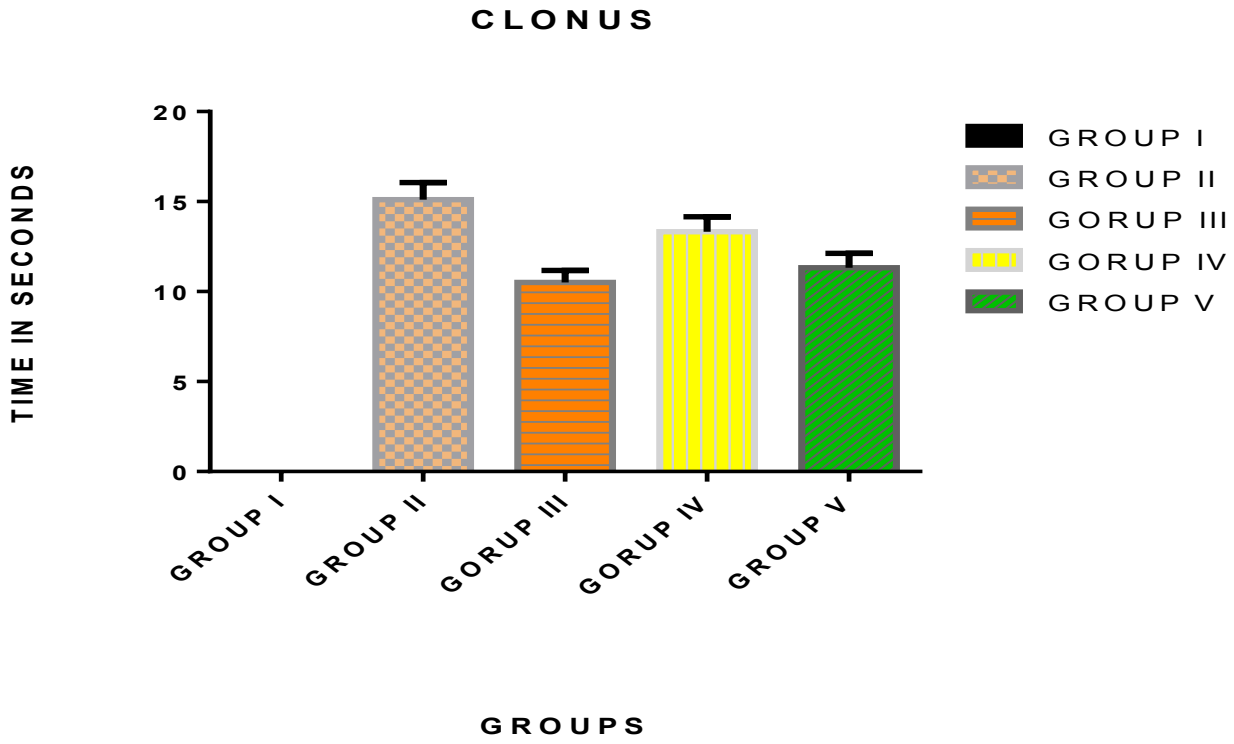


Figure 14:

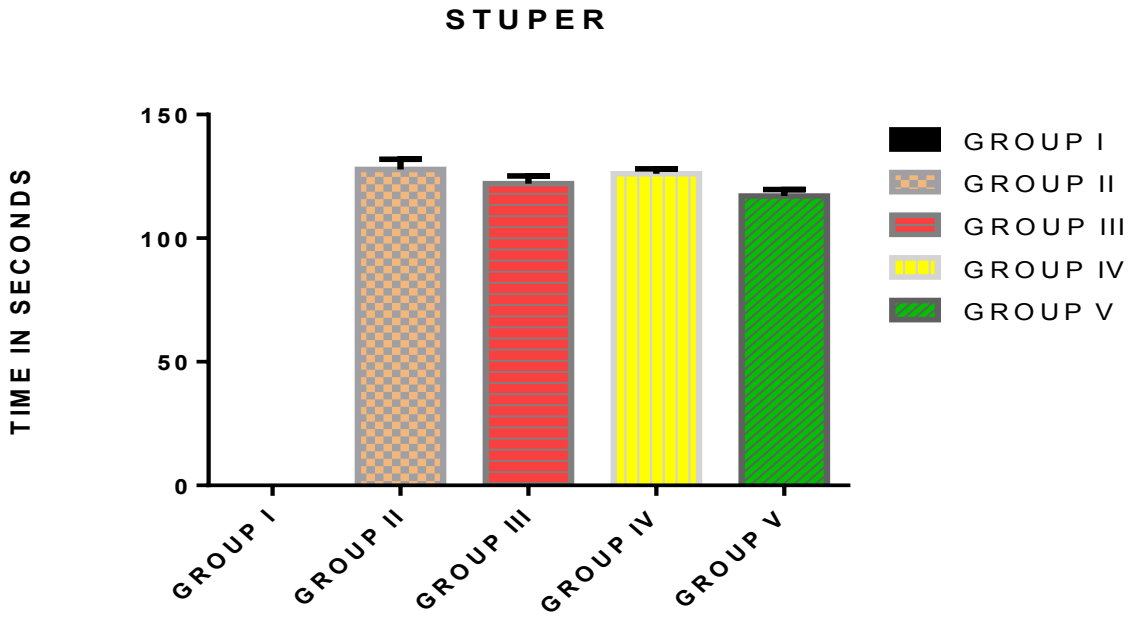


Figure 15:

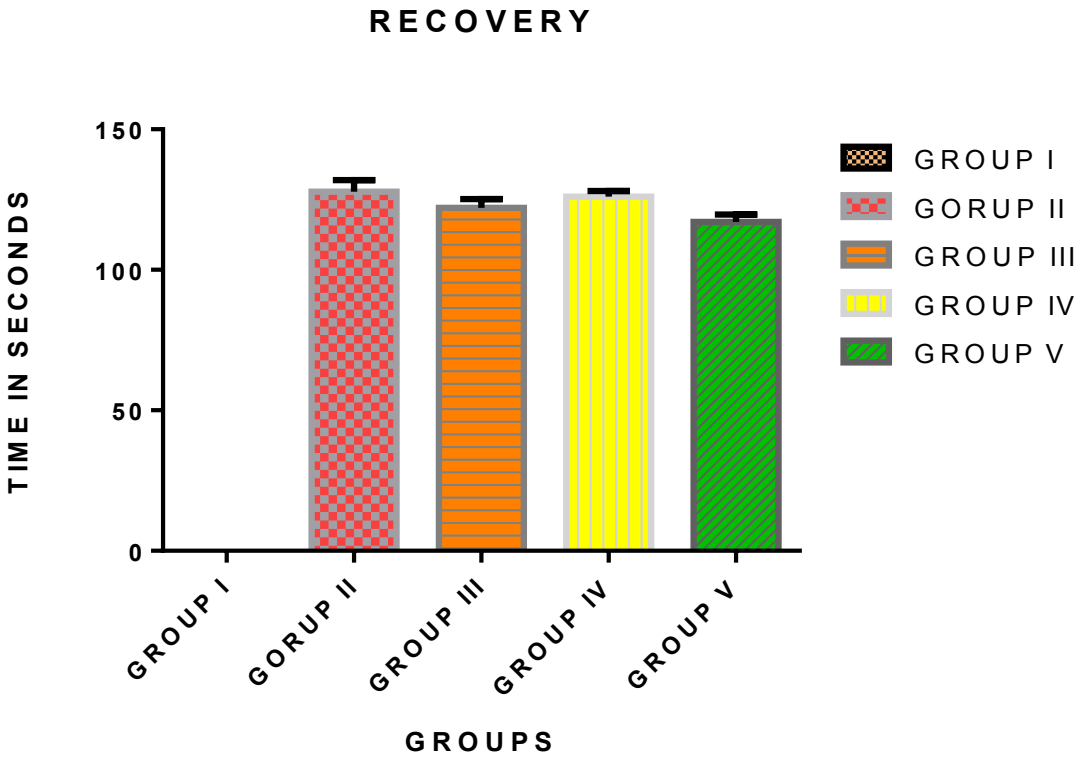


Figure 16:

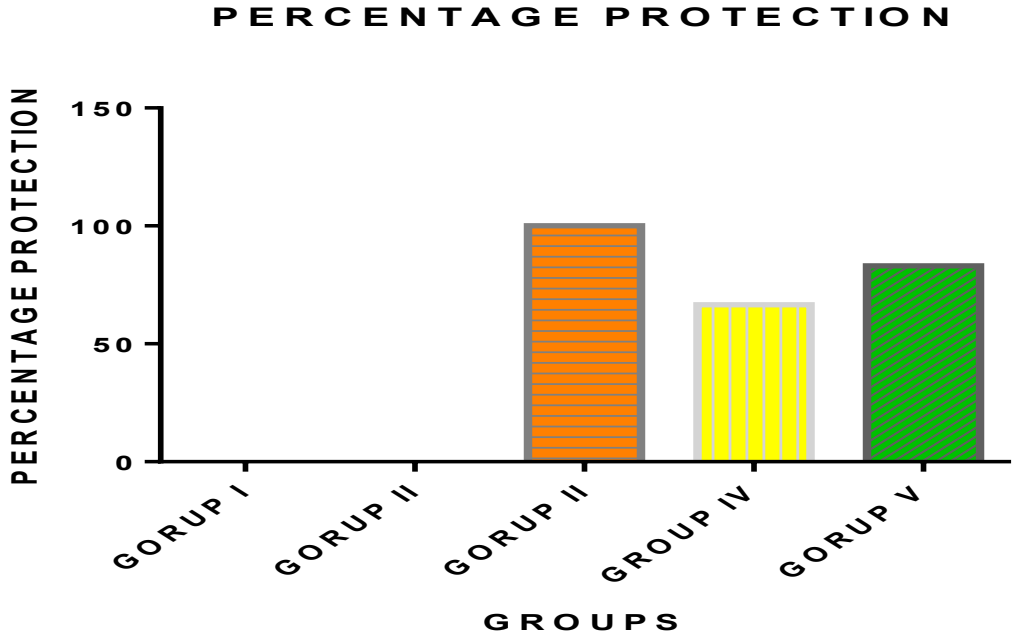


TABLE: 9 EFFECT OF EECA ON LEVELS OF GABA IN MES INDUCED CONVULSIONS

GROUPS	TREATMENT	GABA (ng/mg tissue)
GROUP I	Control	328.3±1.06
GROUP II	MES (60 Hz alternating current of 45mA intensity for 0.2 sec)	231.5±2.67***
GROUP III	PHENYTOIN 25mg/kg p.o. + MES	312.9±1.55***
GROUP IV	EECA 100mg/kg p.o. + MES	285.7±3.23***
GROUP V	EECA 200mg/kg p.o. + MES	293.1±3.17***

Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

a - Group I vs. II, III, IV and V, b - Group II vs. III, IV and V, c- Group III vs. IV and V.

Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.

Figure 17:

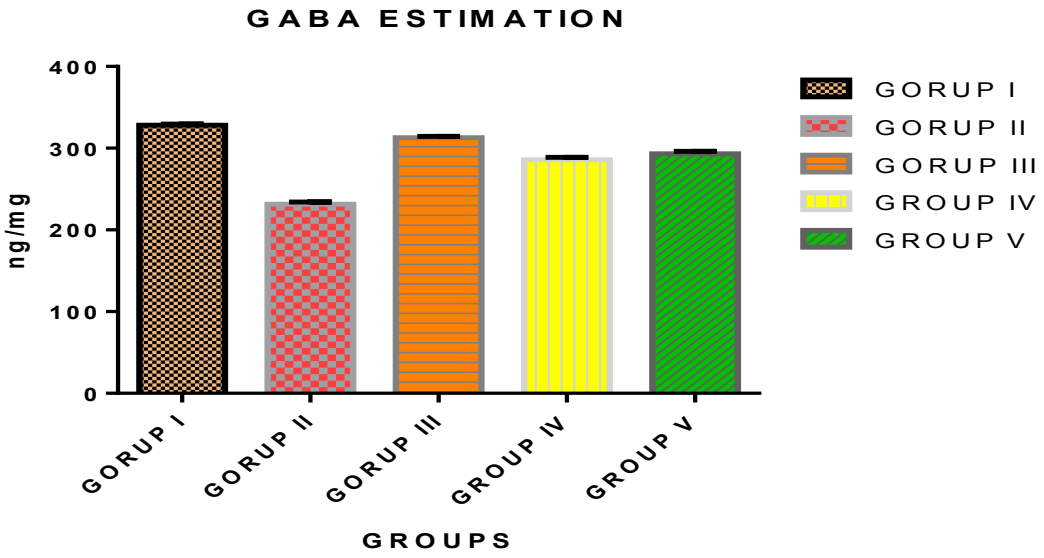


TABLE: 10 EFFECT OF EECA ON ISONIAZID INDUCED CONVULSIONS

GROUPS	Latency (onset of epileptic seizure in sec)	Status of animal after 30min (no. of animals alive)	Status of animal after 24 hr (no. of animals alive)	Percentage protection (in %)
GROUP I	0	0	0	NIL
GROUP II	85±3.12 a***	1	0	0
GROUP III	Nil	3	3	100
GROUP IV	128±2.43 b***c***	2	1	69
GROUP V	137±1.28 a***c***	3	2	83

Values are expressed as mean ± SEM of 6 animals.

Comparisons were made between the following:

a - Group I vs. II, III, IV and V, b - Group II vs. III, IV and V, c- Group III vs. IV and V.

Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant.

Figure 18:

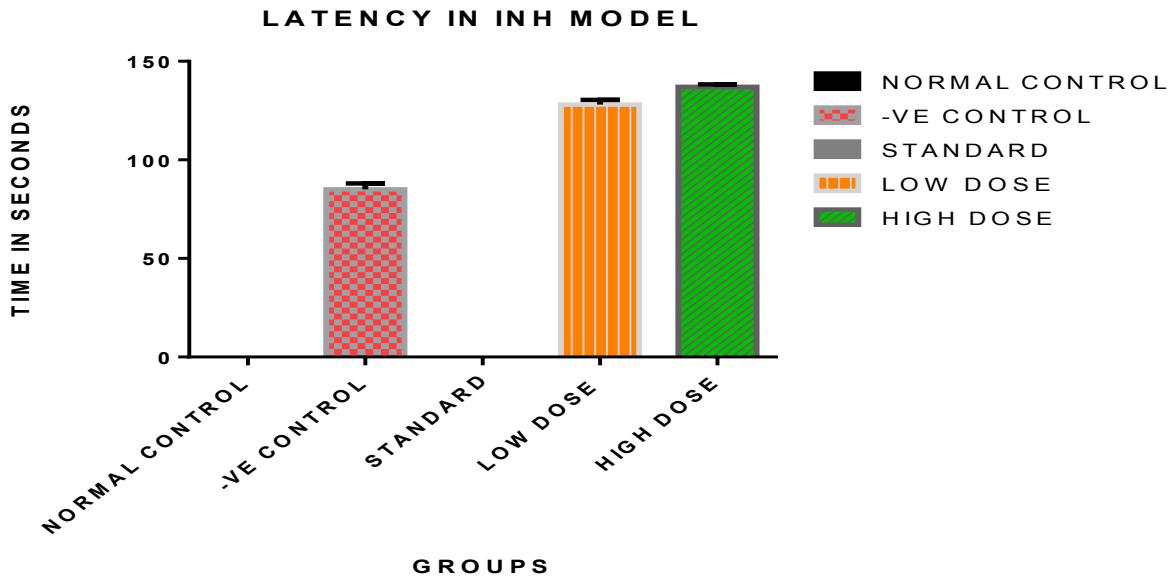


Figure 19:

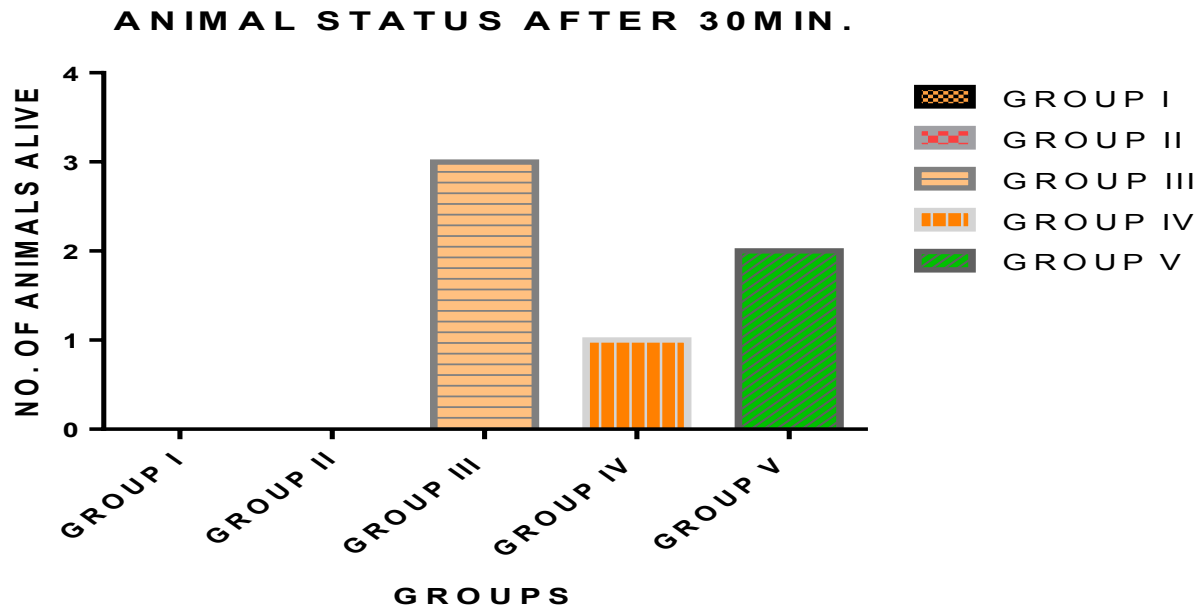
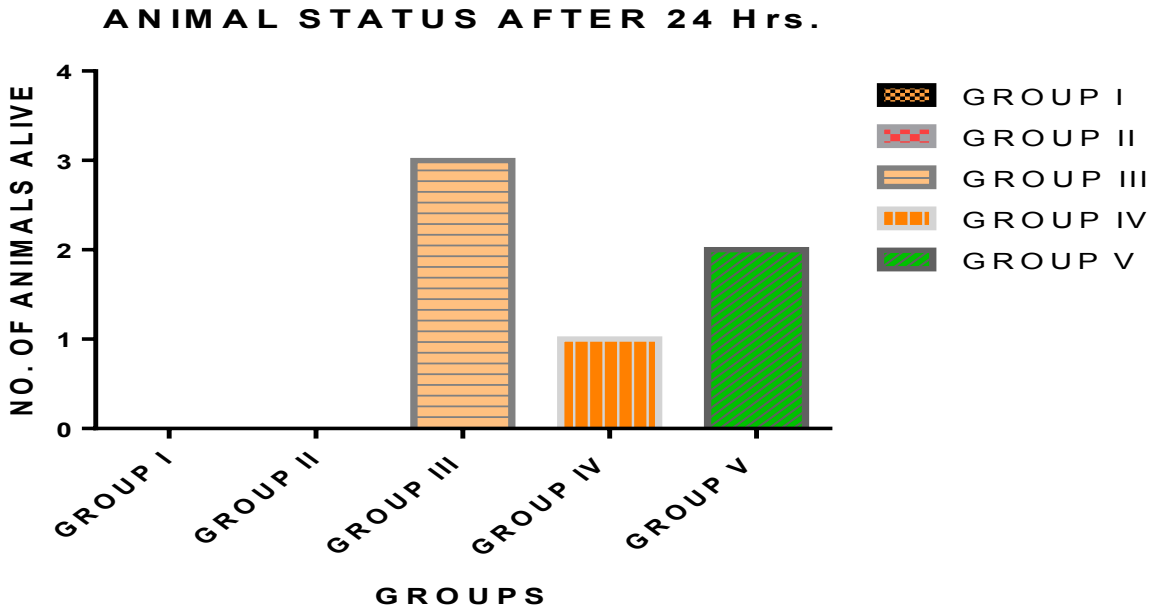


Figure 20:



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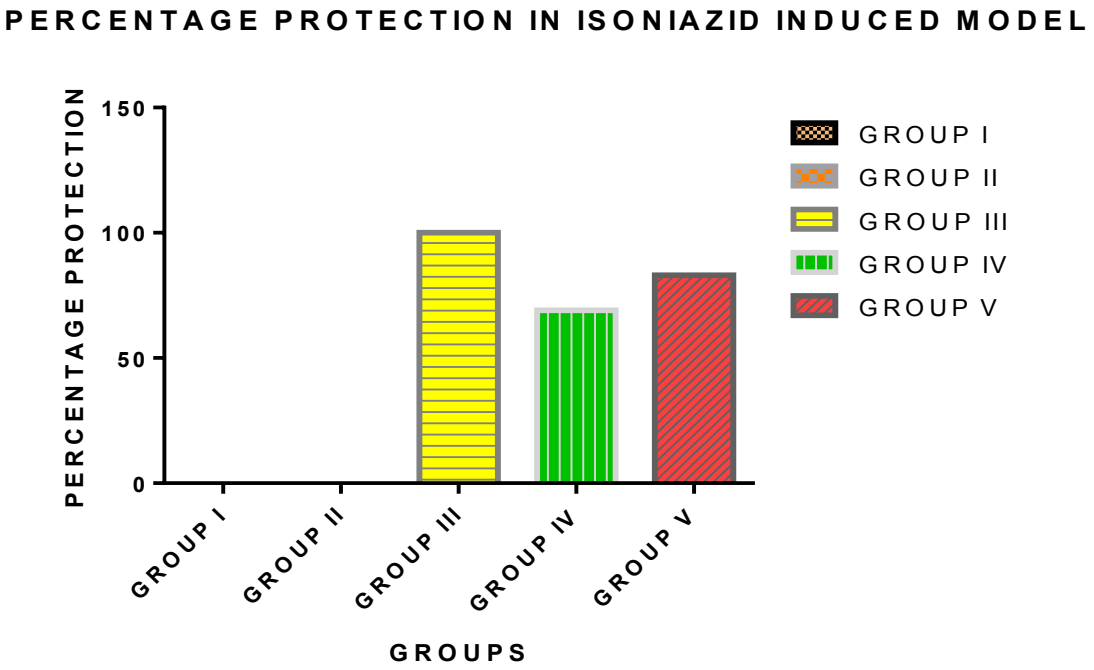
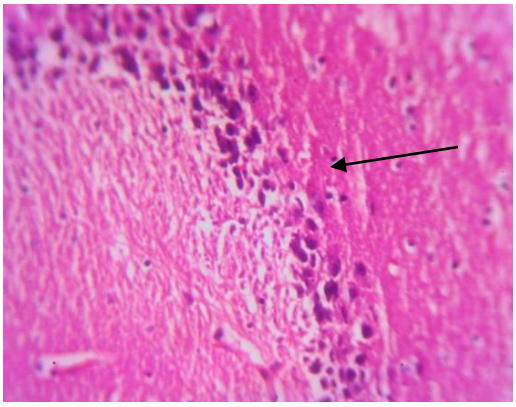
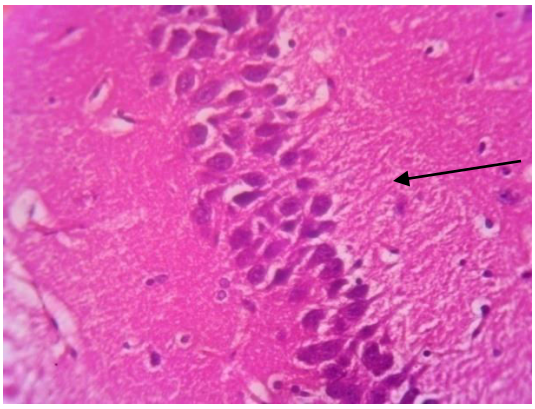


Figures 22: HISTOPATHOLOGY OF HIPPOCAMPAL REGION OF BRAIN

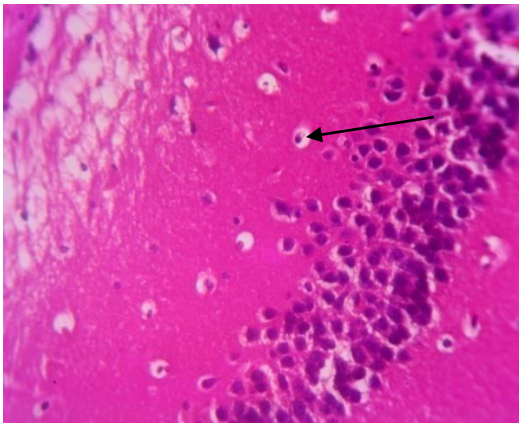
GROUP I



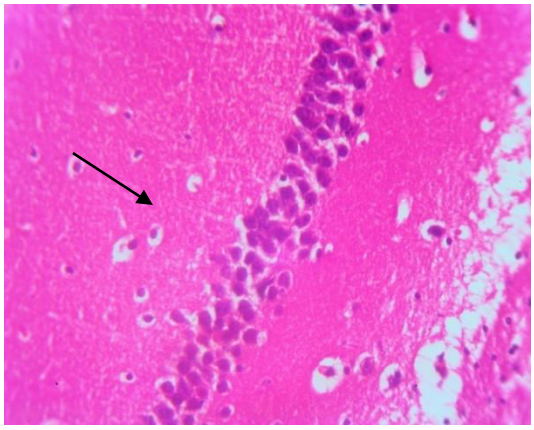
GROUP II



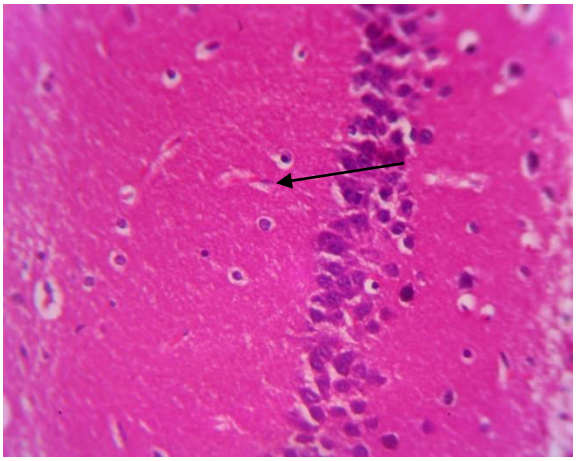
GROUP III



GROUP IV



GROUP V



8. DISCUSSION

Epilepsy is an assortment of different types of seizures originating from several mechanisms that have in common the sudden excessive discharge of cerebral neurons. It may result in a. Loss of consciousness, b. Atypical or odd behaviour, c. distorted perceptions.

Seizures can last from a few seconds to a few minutes[100]. Patients and health care professionals do not always recognize the signs or symptoms, which can include convulsions, a loss of consciousness, blank staring, lip smacking, or jerking movements of the arms and legs[101]. A seizure has a clear beginning, middle, and end.

Epilepsy was one of the first brain disorders to be Described[102]. Epileptic seizures are manifested by an abnormal, excessive, and hyper synchronous electrical discharge of neurons in the brain[103]. Each distinct form of epilepsy has its own natural history and response to treatment[101]. This diversity probably reflects the many different underlying causes of epilepsy and the variety of epilepsy syndromes in which the clinical and pathological characteristics are distinctive and suggest a specific under lying etiologic mechanism[104].

The International League against Epilepsy (ILAE) published a modified version of the International Classification of Epileptic Seizures (ICES), which has continued to be a very useful system[104]. This system is based on the clinical features of seizures and associated EEG findings. The etiology or cellular substrate is not considered. There are three main types of seizures: partial, generalized, and unclassified.

There are many kinds of seizures, each with characteristic behavioral changes and electrophysiological disturbances that can usually be detected in scalp electroencephalographic recordings[103]. A seizure is a transient epileptic event, indicating a disturbance in brain function[101]. Having a single seizure does not necessarily mean that a person has epilepsy[103, 104]. Ten percent of adul ts experience a seizure sometime during their lifetime[105].

Globally, an estimated 2.4 million people are diagnosed with epilepsy each year. In high-income countries, annual new cases are between 30 and 50 per 100000 people in the general population. In low and middle-income countries, this figure can be up to two times higher. In many parts of the world, people with epilepsy and their families suffer from stigma and discrimination.[102]

Epileptic seizures arise from an excessively synchronous and sustained discharge of a group of neurons. The single feature of all epileptic syndromes is a persistent increase of neuronal excitability. Abnormal cellular discharges may be associated with a variety of causative factors such as trauma, oxygen deprivation, tumors, infection, and metabolic derangements. However, no specific causative factors are found in about half of the patients suffering from epilepsy. Underlying causes and pathophysiological mechanisms are (partially) understood for some forms of epilepsy, e.g. epilepsies caused by disorders of neuronal migration and monogenic epilepsies. For several other types of epilepsy, current knowledge is only fragmentary.

Both neurotransmitter systems and ion channels play a crucial role in neuronal excitability[106].

The major developmental disorders giving rise to epilepsy are disorders of neuronal migration that may have genetic or intrauterine causes[107]. Abnormal patterns of neuronal migration lead to various forms of agyria or pachygyria whereas lesser degrees of failure of neuronal migration induce neuronal heterotopia in the subcortical white matter. Recent experimental data suggest that cortical malformations can both form epileptogenic foci and alter brain development in a manner that diffuse hyperexcitability of the cortical network occurs[108]. Other studies revealed increases in postsynaptic glutamate receptors and decreases in g-aminobutyric acid receptors in microgyric cortex which could promote epileptogenesis[109].

The GABA hypothesis of epilepsy implies that a reduction of GABA-ergic inhibition results in epilepsy whereas an enhancement of GABA-ergic inhibition results in an anti-epileptic effect [110]. Inhibitory postsynaptic potentials (IPSPs) gradually decrease in amplitude during repetitive activation of cortical circuits. This phenomenon might be caused by decreases in GABA release from terminals, desensitization of GABA receptors that are coupled to increases in Cl^- conductance or alterations in the ionic gradient because of intracellular accumulation of Cl^- [111]In case of intracellular accumulation of Cl-, passive redistribution is ineffective.

Moreover, Cl—K^+ co-transport becomes less effective during seizures as it depends on the K^+ gradient. As Cl—K^+ co-transport depends on metabolic processes, its effectiveness may be affected by hypoxia or ischemia as well. These mechanisms may play a critical role in ictogenesis and interictal- ictal transition. Several studies have shown that GABA is involved in pathophysiology of epilepsy in both animal models and patients suffering from epilepsy.

GABA levels and glutamic acid decarboxylase (GAD) activity were shown to be reduced in epileptic foci surgically excised from patients with intractable epilepsy and in CSF of patients with certain types of epilepsy[112]. In stiff-man syndrome, a disease associated with epilepsy and diabetes mellitus, auto-antibodies to GAD were demonstrated[113]. A reduction of 3H-GABA binding has been reported in human brain tissue from epileptic patients whereas PET studies demonstrated reduced benzodiazepine receptor binding in human epileptic foci[114]. The degree of benzodiazepine receptor reduction showed a positive correlation with seizure frequency.

The GABA receptor complex is involved in various animal models of epilepsy as well. Low CSF levels of GABA were revealed in dogs with epilepsy[115]. Reduced GAD levels were revealed in the substantia nigra of amygdala-kindled rats[116]. Significant alterations in GABA and benzodiazepine binding have been shown in the substantia nigra of genetically seizure-prone gerbils[117]. Mice with a genetic susceptibility to audiogenic seizures have a lower number of GABA receptors than animals of the same strain that are not seizure prone[118]. Several endogenous (guanidino compounds) and exogenous (e.g. bicuculline, picrotoxin, penicillin, pilocarpine, pentylenetetrazol) convulsants inhibit GABAergic transmission through inhibition of GABA synthesis or through interaction with distinct sites at the postsynaptic $GABA_A$ receptor[110, 119, 120].

Convulsant agents that block synaptic GABA-mediated inhibition, amplify the dendritic spike-generating mechanism that involves Ca^{2+} [121,122]. Synaptic inputs are thought to trigger and synchronize this process throughout a population of cells which then might result in an epileptic fit. Several AEDs are GABA analogues, block GABA metabolism (e.g. vigabatrin, tiagabine, and valproate) or facilitate postsynaptic effects of GABA. However, a study evaluating dose-dependent behavioral effects of single doses of vigabatrin in audiogenic sensitive rats, suggests that the antiepileptic properties of vigabatrin not only depend on GABA-ergic neurotransmission but might also be explained by decreased central nervous system levels of excitatory amino acids or increased glycine concentrations[123].

Glutamatergic synapses play a critical role in all epileptic phenomena. Activation of both ionotropic and metabotropic postsynaptic glutamate receptors is proconvulsant. Antagonists of N-methyl-D aspartate (NMDA) receptors are powerful anti-convulsants in many animal models of epilepsy.

Abnormalities of CNS catecholamines have been reported in several genetic models of epilepsy. In spontaneous epileptic rat, dopamine was decreased in the nucleus caudatus whereas noradrenaline was increased in midbrain and brainstem[124]. Decreased levels of dopamine have been found in epileptic foci of epilepsy patients[125]. In animal models of absence epilepsy, seizures are exacerbated by dopamine antagonists while fits are alleviated by dopamine agonists[126]. These results suggest that decreased dopamine facilitates appearance of seizures by lowering the threshold triggering such seizures.

Periventricular heterotopia is an Xlinked dominant disorder of cerebral cortical development. Fox *et al.* (1998) showed that mutations in the filamin 1 gene prevent migration of cerebral cortical neurons causing periventricular heterotopy[127].

Affected females present with epilepsy whereas affected males die embryonically. Recently, however Recently, however, a male patient with bilateral periventricular and subcortical heterotopia was described which raises the possibility of a novel gene involved in brain formation [128]. X-linked lissencephaly and double cortex syndrome is another disorder of neuronal migration. Double cortex or subcortical band heterotopias often occurs in females whereas more severe lissencephaly is found in affected males. A causal mutation in a gene called doublecortin was recently identified[129]. It was suggested that doublecortin acts as an intracellular signaling molecule critical for the migration of developing neurons[130]. Although these disorders are relatively rare, studying the underlying pathophysiological mechanisms may shed light on the pathophysiology of more common epileptic syndromes.

About 40% of patients suffering from epilepsy have a genetic background that contributes to the aetiology of epilepsy[131]. Most familial epilepsies like juvenile myoclonic epilepsy, childhood absence epilepsy, and benign childhood epilepsy with centrotemporal spikes have a complex mode of inheritance resulting from the interaction of several loci together with environmental factors[132]. In patients with absence seizures (and their first degree relatives), biochemical changes (e.g. increased plasma glutamate levels) have been identified which can be related to a generalized increase in cortical excitability[133].

Probably, the genetic predisposition of absence epilepsy is based on a gene-dependent biochemical derangement leading to increased cortical excitability. Genetic data generated by studies on animal models of absence epilepsy show a relative simple inheritance factor of one gene that determines being epileptic or not while other genes determine number and duration of

epileptic fits[134]. Monogenic epileptic disorders are rare, accounting for no more than 1% of patients. Recent advances in the genetics and molecular biology of these diseases unraveled the underlying Pathophysiology of some of these epileptic syndromes[135].

Although various epileptic syndromes were shown to differ pathophysiologically, they apparently share common ictogenesis-related characteristics such as increased neuronal excitability and synchronicity. Emerging insights point to alterations of synaptic functions and intrinsic properties of neurons as common mechanisms underlying hyper excitability. Progress in the field of molecular genetics revealed arguments in favor of this hypothesis as mutations of genes encoding ion channels were recently discovered in some forms of human epilepsy.

In most cases epilepsy treated with medications. Over the past decades new drugs for epilepsy have became available which allow many people with epilepsy to live virtually seizure free lives. Anti-epileptic drugs do not cure the epilepsy they only control it[136]. Most anti-epileptic drugs exert their anti-epileptic properties through only a few neurochemical mechanisms that are meanwhile basic pathophysiological mechanisms thought to cause seizures. Pathophysiology of epilepsy and its underlying histological and neurochemical alterations has contributed to rational development strategies of new anti-epileptic drugs (AEDs).

Antiepileptic drugs that are presently in clinical use include phenytoin, carbamazepine, ethosuximide, phenobarbitone, tiagabine, vigabatrin, gabapentin and clonazepam are the major drugs used for the treatment of epilepsy. The drug anti-epileptic drug phenytoin acts by blockade of voltage dependent sodium channels and stabilizes the neuronal membrane. It inhibits the generation of repetitive action potentials. Voltage dependent Na^+ channels enters an inactivated state and deley the recovery of these channels from inactivation. AEDs have prominent side effects and fail to alter the course of epileptic complications. Dyskinesia, gingival hypertrophy, macrocytic anaemia, dermatitis, thyroiditis, taste disturbance, loss of appetite, dizziness, headache, flushing, increased urine output, gastro intestinal disturbance, skin rashes, drowsiness, over growth of hair, acne, hair loss, constipation, diarrhoea, double vision, insomnia, attention difficulties, visual disturbance, cough, weight changes, abdominal pain are the adverse effect produced, which make more disturbance in medication periods of patients.

Hence herbal drugs as therapeutic agents are preferred to reduce severe adverse effects of the allopathy therapy. Therefore, scientists are on the hunt for newer alternatives, with lesser side effects, self-administrable, less expensive and with complete reversibility. Much of these

properties are observed in drugs of natural plant origin. Globally traditional system of medicine has been used to treat various diseases throughout the human history. Many plants are reported to have anti-epileptic activity. Moringa oleifa*(Moringaceae)*[137], *Acorus calamus* (Araceae)[138], *American ginseng* (Araliaceae)[139, 140] , *Delphinium denudatum* (Ranunculaceae)[141-145] , *Nardostachys jatamansi* (Valerianaceae)[146, 147], *Herpestis monniera* (Scrophulariaceae)[148, 149, 150] , *Ambrosia paniculata* (Asteraceae)[151] are the some of the plants having anti-epileptic activity.

In the present study the EECA was evaluated for its anti-epileptic activity in experimental mice. *Cassia alata* Linn. used as purgative, expectorant astringent, vermicide and to treat all skin diseases. *Cassia alata* is one of the species that is now increasingly being used by herbalists as laxative, abortifacient; and in the treatment of various skin and respiratory diseases. The leaves of *Cassia alata* Linn. have been qualitatively analyzed for the presents of primarily five pharmacologically active anthraquinones: rhein, aloe-emodin, chrysophanol, emodin, and physcion[152], as well as the flavonoid kaempferol[153]. Rhein and chrysophanol are known to be present in the roots[154], in addition to two other quinine pigments[155], These anthroquinone derivatives are well known to exhibit a variety of biological activities[156], such as antimicrobial[154], cytotoxic[157], etc. They are also claimed to possess other medicinal properties, hence their use by traditional healer in folk medicine.

Acute oral toxicity studies revealed the non-toxic nature of the *Cassia alata* Linn. There was no lethality found at the dose of 2000mg/kg/p.o. which shows the safety of the plants.

Flexion is the first stage in convulsive epilepsy, where the bending of joints, limbs occur. At this stage the rapid onset of a rigid posture with head flexed forwards, elevation of both arms, and flexion of the trunk forwards at the thigh. EECA treated animal shows significantly decreased in flexion period when compared to the untreated group.

Extensor is the next stage followed by flexion, were limb extension occurs. Both flexion and extensor occur at very short duration. EECA treated animal shows significantly decreased in extensor period when compared to the untreated group.

Clonus stage in seizures consist of bilaterally synchronous involuntary muscle jerks that results in singly or in a brief salvo of repeated jerks. EECA treated animal shows significantly decreased in clonus period when compared to the untreated group.

The maximal electroshock induced seizures produce repetitive stimulation of high frequency action potentials thus opening of Na^+ channels and increasing Ca^{2+}intracelluraly leading depolarisation of cell. It has been found out that treatment of mice with *Cassia alata* Linn. showed significant decrease in the hind limb extensor period.

Animal models of seizures induced by electrical stimulation convey the advantage of reproducing epileptogenic features in the intact brain with low mortality and high reproducibility. Moreover, unlike chemical-induced seizures, postictal alterations from electrical stimulation can be investigated when the epileptogenic cause is no longer present. However, seizure modelling by electrical stimulation does not provide cell-type specificity in the brain. In addition, stimulation protocols can be costly and laborious when used for chronic studies[158].

GABA is an inhibitory neurotransmitter and glutamate is an excitatory neurotransmitter which is responsible for the production of excitation of neurons thus plays an important role in the generation of seizures. The Maximal Electro Shock (MES) induced seizure showed significant decreased levels of GABA in brain, thus showing that GABA plays an important role in the inhibition of seizures i.e., The percentage protection in the EECA with MES treated animal is significantly increased when compared to MES treated alone animal groups. GABA level in EECA with MES treated group is significantly increased when compared to MES treated alone animal group.

Rodents with spontaneous recurrent seizures have been generated by using chemo-convulsants, primarily pilocarpine and kainic acid[159]. Usually, these models intend to mimic TLE (Temporal Lobe Epilepsy), and therefore rodents must display a similar “clinical history” as the human counterpart, including an initial precipitant injury afflicting the hippocampus and/or the temporal lobe (eg, status epilepticus [SE]), a latent period between the injury and the occurrence of spontaneous seizures, chronic manifestation of spontaneous seizures (usually partial and tonic-clonic seizures), and histopathological changes deemed characteristic of TLE.[157,160,161]

Inorder to study the Temporal Lobe Epilepsy and Status Epilepticus the INH induced epilepsy models are chosen. The compound is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are elicited in mice which is due decreasing in the pyridoxine metabolism. Due to decrease in the pyridoxine level, it produce the convulsion in mice. The EECA and INH treated

animals showed significant decrease in the onset and decreased duration of the seizure when compared with INH alone treated group animals. With that increased percentage protection from epilepsy in INH model shows that plant having anti-epileptic activity which can be used in TLE.

The presence of Flavonoids may helps in the anti-epileptic property of Cassia alata Linn. The histopathological study of hippocampal region of brain shows that there is increased neuronal density produced by the EECA with MES treated groups compared to the MES alone treated group. The increased activity of GABA in EECA treated group may be due Inhibition of GABA transaminase enzyme or Inhibition of GABA transporter.

And due to its anti-oxidant activity of *Cassia alata*, helpful to protect brain from toxicity, which can help from seizures and other epilepsy conditions[77].

9. CONCLUSION:

The leaves of *Cassia alata* Linn. showed reduction in the flexion, extensor, clonus and stuper duration in MES induced epilepsy model. So the drug can be used in grandmal epilepsy. In INH model latency of epilepsy and lethality of animal was reduced. So the drug can be used in TLE.

The histopathology of hippocampal region of brain showed normal architecture and there is increased neuronal density which is comparable with phenytoin.

GABA level is increased, so the drug probably acts as GABA mechanism either Inhibition of GABA transaminase enzyme or Inhibition of GABA transporter.

Thus, it may be concluded that Cassia alata Linn. produces significant anti-epileptic activity in both MES and INH induced epilepsy in mice, which is comparable with that of phenytoin. Further work is necessary to elucidate the mechanism of action involved in the anti-epileptic activity of Cassia alata Linn. with special reference to Phytochemical constituents.

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